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The First Living Systems: a Bioenergetic Perspective

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INTRODUCTION

> "Cellular organization, far from an afterthought, must have been from the beginning part and parcel of the origin of life. . . Therefore a believable biopoetic scheme is one that creates mounting levels of biological order naturally, by providing the means to convert the flux of energy into the organization of matter. This seems to me inconceivable without compartments."
>
> Franklin Harold, 1986

This review will address the question that is implicit in the above quotation: did the living state necessarily arise a priori from preexisting cellular structures that were required to capture energy for growth processes? Or, as is more generally believed, were there noncellular macromolecular precursors that we could identify as being alive, with cellular life developing at a later evolutionary stage?

It is not difficult to argue that research on the beginning of life represents one of the last bastions of classical science, defined by the significance of its central goal, its breadth of scope, and a ratio of hypothesis to fact approaching infinity. It is also characterized by a relative paucity of research funding, which in turn is reflected by the number of investigators. Societies of molecular biologists, biochemists, and neuroscientists count their membership in the ten of thousands, while the International Society for the Study of the Origin of Life has 400 members.

Despite the minuscule number of investigators, there is no doubt that a plausible answer to the question of how life began will be a major step forward in the biological sciences, as demonstrated by the worldwide reaction to the possibility that the origin of terrestrial microbial life may have had a counterpart on Mars (93). This evidence was obtained from a meteorite (ALH84001) that was ejected into an Earth-crossing orbit during a giant impact on Mars about 16 million years ago and fell in Antarctica about 13,000 years ago. The meteorite is

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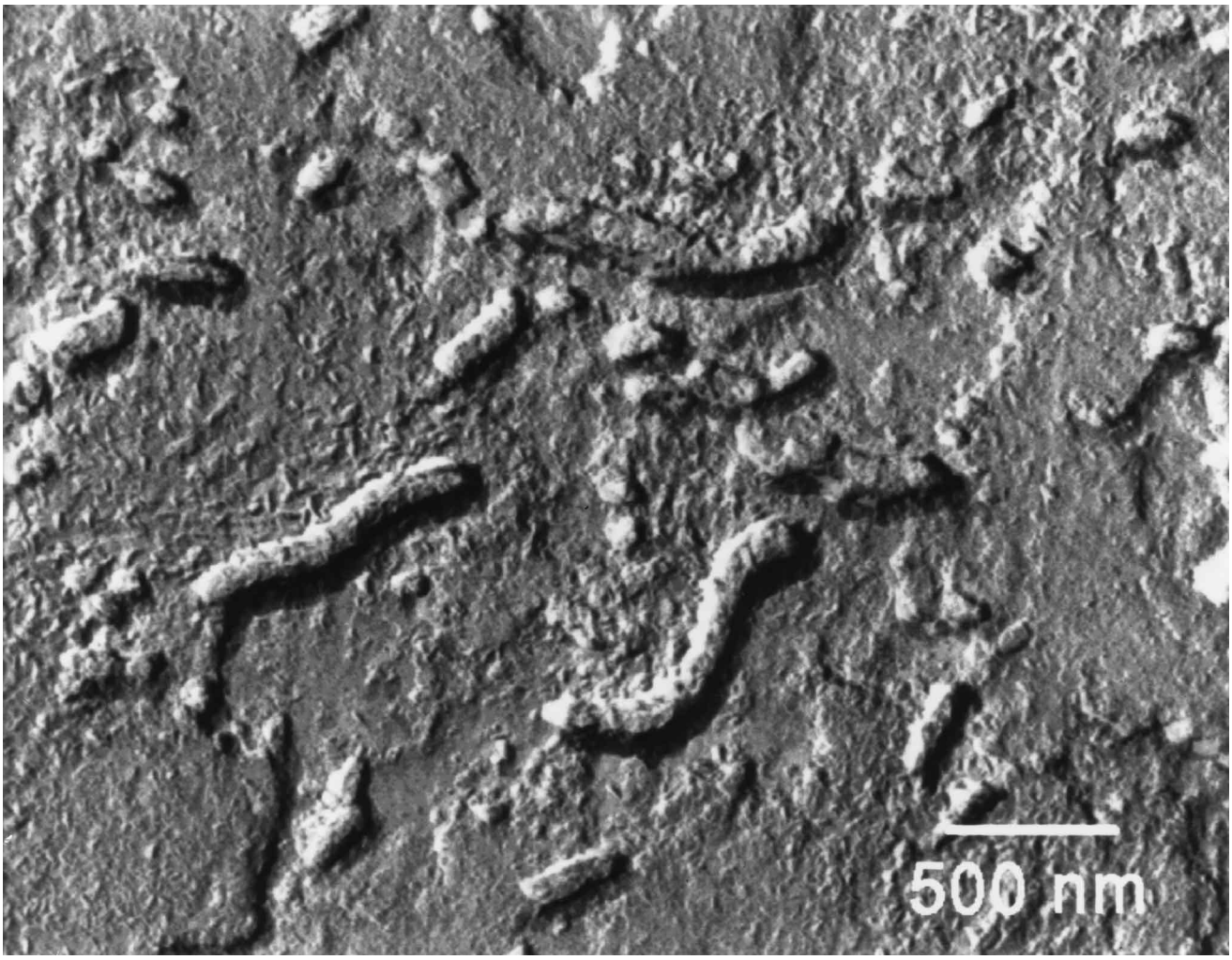

FIG. 1. Electron microscopic image obtained from a platinum-carbon replica of an etched mineral surface in the Antarctic meteorite ALH84001, believed to represent a fragment of the Martian crust produced by an impact event approximately 16 million years ago. The surface reveals structures 700 nm long and 100 nm in diameter which resemble terrestrial nanobacteria. The same meteorite contains traces of PAHs, unusual carbonate deposits, and magnetite crystals similar to fossil remains of bacterial magnetosomes. This evidence led McKay et al. (93) to propose that microbial communities existed on Mars over 3.6 billion years ago. Courtesy of the National Aeronautics and Space Administration.

probably derived from the Martian crust at a depth of several hundred meters, and it contains carbonate inclusions about 3.6 billion years old. Minerological features of the carbonate can be interpreted as having a biogenic origin, and organic molecules in the form of polycyclic aromatic hydrocarbons are present as well. Perhaps most intriguing are scanning electron micrographs of microscopic fissures in the carbonate, which show globular structures that have been interpreted as possible microfossils (Fig. 1). These resemble terrestrial nanobacteria that have recently been found in copper sulfide deposits (140).

This evidence, taken together, suggests that microbial life may have existed on Mars at about the same time as it developed on the Earth. However, strong counterarguments can be made (1a), and it is clear that we are several years away from a definitive answer. But if ALH84001 ultimately turns out to contain fossil remains of microbial communities, what was the source of energy and nutrients used by microorganisms existing deep in the Martian crust? A convincing answer to this question would help constrain the many possible ways that life could have begun on the Earth.

ENERGY FLOW AND THE ORIGIN OF LIFE

A variety of perspectives can be used to guide research approaches related to the origin of life, and they have been addressed in previous reviews (2, 15, 19, 22, 25, 26, 35, 45, 53, 68, 71, 84, 85, 89, 90, 97, 101, 107, 109, 110, 114, 131, 135). The temporal perspective concerns when life began, and the best evidence from the fossil record suggests that microbial communities were forming stromatolites at least 3.5 billion years ago (129–131). The geophysical perspective asks how the origin of life was constrained by the physical environment at that time. The early Earth apparently was not a benign environment, and life may have sprung up on more than one occasion, only to be extinguished by giant impactors that sterilized the Earth's surface and vaporized the oceans (91, 143). A third perspective concerns the chemistry of the prebiotic Earth: which organic monomers were plausibly available (23, 98), and how did they assemble into complex systems that led to the first molecular assemblages able to reproduce their own structure (85)? Yet another perspective is related to the information

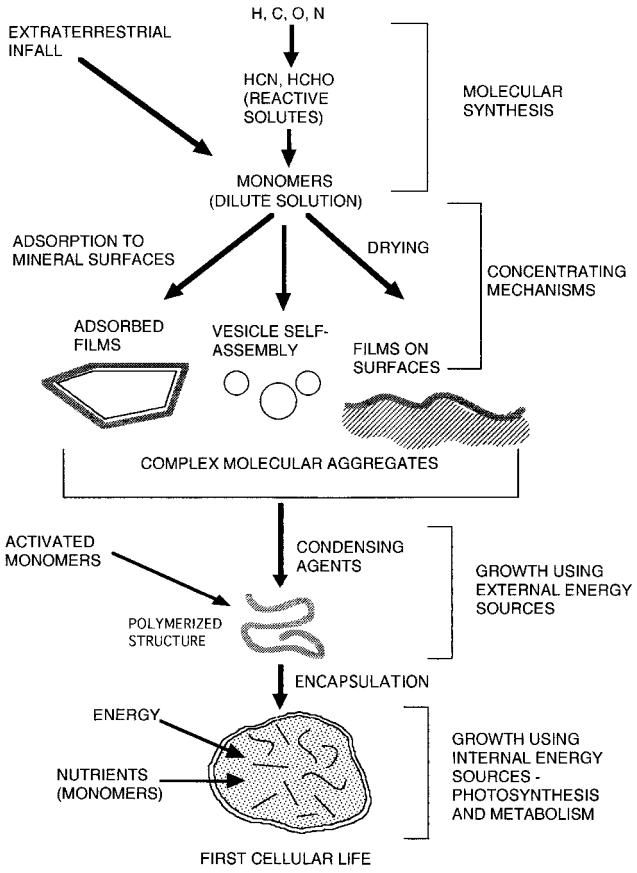

FIG. 2. Energy input into prebiotic evolutionary processes related to the origin of cellular life (see the text for details).

content of the living state: what kinds of information-bearing polymers could have arisen from the mixture of organic molecules present on the early Earth, and how did they transmit information through a cycle of growth and replication (71, 110)?

All of these approaches are necessary for a full understanding of the origin of life and will be touched on in this review. However, the primary focus will be the energy sources available to drive the processes leading to the first forms of life. The use of energy flow as a perspective has certain aspects that should be clarified before going on, particularly in defining the levels of organization related to energy sources involved in prebiotic evolution (Fig. 2).

The first level is the energy required to drive the synthesis of reactive molecules like formaldehyde and hydrogen cyanide, which then have the chemical potential to produce more complex molecules such as amino acids, sugars, and purines (128). These reactions require relatively high energies, modeled in the laboratory by electrical discharge and UV light. The second level is the energy required for concentrating dilute solutions of potentially reactive solutes. Concentration could occur by adsorption to mineral surfaces, simple drying at intertidal zones, or self-assembly processes producing molecular complexes in the form of films and bilayer vesicles. The third level is the input of external energy that would drive polymerization reactions in a nonliving assembly of molecules, ultimately increasing the chemical complexity of the system. The fourth level is the energy made available by internalized chemical reactions in a cellular microenvironment. Any replicating molecular system operating at this level of energy utilization would approach the definition of the living state. We will focus here on the last two levels, that is, mechanisms by which external and internal energy sources could drive reactions in complex assemblies of organic molecules on the evolutionary pathway to cellular life. This is probably the area of our greatest ignorance and is the sense of the quotation from *The Vital Force* that introduces this review (63).

A Thought Experiment

To illustrate how a bioenergetic focus is related to the origin of life, it is interesting to conduct a thought experiment in which we produce a series of increasingly complex simulated prebiotic soups of biochemical monomers, using what we know about the composition of a living cell. The simplest soup will contain millimolar concentrations of the monomers of proteins (20 or so amino acids) and the monomers of polysaccharides and polynucleotides, including glucose, ribose and deoxyribose, two purines (adenine and guanine), three pyrimidines (cytosine, thymine, and uracil), and phosphate. It will also contain a mixture of fatty acids and glycerol that potentially can assemble into lipid molecules. Finally, we can add salts and trace elements that were likely to have been available in the prebiotic milieu.

The soup is first deaerated by passing carbon dioxide through for some time to remove oxygen, which otherwise would oxidize some of the molecules and initiate damaging free radical reactions. This step also simulates the carbon dioxide atmosphere considered to have prevailed on the early Earth. After sterilizing the flask and its contents, we place it in an incubator and wait to see what happens, recognizing that Louis Pasteur performed similar experiments and reported his results in 1864 (118).

The first noticeable change occurs within minutes as the solution cools from the sterilization temperature and the fatty acids aggregate to form a milky suspension of micellar and vesicular structures. Over a period of several hours, the suspension darkens due to the browning reaction that occurs between the carbohydrates and amino acids in the mixture, but afterward little further change occurs. Why is this? The answer has two related aspects. First, the mixture is disordered: there are no structures present to provide a source of order, although it is possible that the lipid assemblies and perhaps the inner glass surface of the flask have modest ordering effects. Second, the flask and its contents contain no free energy beyond the small content of chemical energy available in the dissolved solutes. It is virtually at equilibrium.

To take the thought experiment further, we might attempt to overcome the lack of order. In one prebiotic scenario, this could be accomplished by adding a complex mineral surface, such as clay, as first suggested by Bernal (12) and later elaborated by Cairns-Smith (17), who proposed that mineral surfaces could potentially act as templates for assembling organic solutes into orderly arrays. If clay is added to our prebiotic soup, there is no doubt that the mineral surfaces would adsorb a variety of the polar solutes with considerable specificity (51). Furthermore, under certain conditions, the clay can act as a catalyst, as discussed below. However, a living system of molecules would not be expected to appear upon addition of mineral surfaces. Even though some ordering of organic molecules would occur on the surfaces, there is still no source of chemical energy to drive the reaction.

As a next step, we can include a source of free energy. There

are several ways to do this. For instance, we might add pyrophosphate bond energy in the form of nucleoside triphosphates or pyrophosphate itself (6–8). We would discover that this is a relatively short-lived source of energy, since hydrolysis of the pyrophosphate bonds would soon return the solution to equilibrium. Even though the chemical energy in pyrophosphate could potentially phosphorylate organic compounds such as the glucose and glycerol that are present, no catalysts are present to direct the reactions toward a primitive metabolic pathway. Another possibility is to add a source and sink of redox potential, for example solutes such as ferrous iron salts or hydrogen sulfide, but this again would quickly move toward equilibrium. We might illuminate the solution, either continuously or at intervals, and now we have a substantial source of free energy. However, no pigments are available—how could the light energy be captured and stored in a useful form? Finally, we could cycle the pH up and down by several pH units. If membranes are present as lipid bilayers, this would also provide a source of free energy in the form of proton gradients, and at least for a few minutes a burst of chemiosmotic potential would be present in the system. Again, in the absence of catalyzed reaction pathways capable of capturing the energy, the gradients would be transient and quickly decay due to leaks in the bilayers.

Before giving up, we could take an extreme step toward adding order and energy to the system. Imagine that on the early Earth, a complete system of catalytic and information-bearing molecules happened by chance to come together in a tide pool that was sufficiently concentrated to produce the equivalent of the contents of our flask. We could model this event in the laboratory by gently disrupting a live bacterial culture, subjecting it to a sterilizing filtration step, and adding the mixture to the flask of nutrient broth. No living cells are present, but entire bacterial genomes are available, together with ribosomes, membranous vesicles, ATP and other energy-containing substrates, and thousands of functional enzymes. Once again, would a simple living system arise under these conditions? Although Kauffman might be optimistic about the possibilities (78), most experimentalists would guess that little would happen other than slow, degradative reactions of hydrolysis, even though virtually the entire complement of molecules associated with the living state is present. The dispersion has lost the extreme level of order characteristic of cytoplasm in contemporary living cells. Equally important is that the ATP would be hydrolyzed in seconds, so that the system still lacks a continuous source of free energy to drive the metabolism and polymerization reactions associated with life.

The thought experiment illustrates the way that energy flow will be considered in this review. The high degree of molecular order and energy metabolism characteristic of life could not spring full-blown into a primitive planetary environment. Instead, a variety of chemical and physical processes could lead to relatively simple molecular assemblies that utilize available energy sources and were plausibly on the evolutionary pathway to the first living cells. Part of the argument here is that certain components of the prebiotic mixture of organic molecules could form microscopic compartments bounded by membrane-like structures and that the compartments could capture energy and make it available for encapsulated systems of replicating macromolecules. If so, it follows that living systems did not necessarily precede membrane encapsulation but would have had access to cellular microenvironments from the beginning of life on Earth.

THE ARCHEAN EARTH

How were free energy sources utilized by the self-assembly processes that led to the first living cells? To take this discussion further, we will first summarize recent advances in our understanding of the primitive Earth environment, particularly those relevant to sources of energy and organic compounds.

(i) The solar system formed approximately 4.6 billion years ago (119, 144). This date is derived from isotope ratios measured in meteorites and is assumed to represent the age of the Earth as well.

(ii) The most plausible origin of the Earth-Moon system involved the collision of a Mars-sized object with the Earth some 4.5 billion years ago, well after the Earth had reached approximately 90% of its current mass (14, 65). The enormous energy released by such a collision would have melted the Earth's crust and degraded any organic compounds that were present ab initio. This means that all of the organic material associated with the origin of life accumulated after the Moon-forming event.

(iii) Both the Moon and the early Earth underwent a late bombardment of asteroid-sized objects until about 3.9 billion years ago (163). Giant impacts in the solar system continue even today, a point made abundantly clear by the Shoemaker-Levy comet collision with Jupiter in July 1994. Lunar craters provide information about the energies associated with such collisions, and it is assumed that the early Earth underwent a similar bombardment. The energy released by impacts in the range of those producing the largest craters on the moon would have been sufficient to partially vaporize the ocean and sterilize the Earth's surface, leading to the concept of impact frustration of the origin of life (57, 91, 143).

(iv) A variety of plausible mechanisms could have synthesized organic compounds from atmospheric components on the early Earth (10, 97, 147), and there is increasing evidence that extraterrestrial delivery of preexisting organic material also played a significant role (23, 24).

(v) The Earth cooled to the point that oceans formed about 4.4 billion years ago, and the cooling trend continued until at some point organic molecules could maintain self-assembled structures against dispersive thermal effects. This temperature was likely to have been in the range now associated with thermophilic microorganisms, and evidence from molecular phylogeny strongly indicates that the earliest cells were most closely related to today's thermophilic bacteria (117, 166).

(vi) The oldest geological formations are Canadian rocks about 4.0 billion years old, and the first convincing microfossils appear in sedimentary deposits about 3.5 billion years old (129–131), suggesting that cellular life with complete genetic systems and a translation apparatus existed at that time. Significantly, this is also the estimated age of the carbonate minerals deposited in ALH84001 (93).

Given the range of global conditions outlined above, it is clear that simplifying assumptions must be made by investigators dealing with the origin of life to constrain a given experimental or theoretical approach. These assumptions are often not made explicit, and at least in this review it is worth taking a moment to outline the most significant assumptions relevant to the primitive Earth environment.

What Scale Is Appropriate for Prebiotic Evolution Scenarios?

Scenarios for the origin of life can focus on global scales, local regions, or microenvironments. The assumption of a global scale requires that organic concentrations in the entire ocean must be taken into account. If this assumption is made,

plausible concentrations resulting from synthetic reactions turn out to be very dilute, in the nanomolar to micromolar range. Even under the most favorable conditions of a reducing atmosphere, global concentrations of amino acids might reach only 100 μM (13, 145). At such concentrations, chemical polymerization reactions proceed slowly if at all, and a second assumption must be made that a concentrating mechanism that could work on the global scale was available. For instance, Bada et al. (4) proposed that the early Earth may have been cold, rather than warm, so that oceans froze. Under such conditions, solutes are concentrated and might then undergo further reactions pertinent to the origin of life.

A local-scale assumption allows virtually any environment to be available, and many of these environments provide sources of energy for concentration and driving chemical reactions. Examples of diverse local environments on the Earth today include ice-free Antarctic deserts that are the closest terrestrial analogs to the surface of Mars, tropical tide pools, geothermally heated springs, and hydrothermal vents. If the origin of life is better understood in terms of a specialized environment rather than a generalized global perspective, it is easy to imagine, with Darwin, that a tide pool is a reasonable contemporary model. Tide pools make energy available as light, heat, and dehydrating conditions, and Keefe and Miller (79) found that such conditions significantly increased the yield of several synthetic reactions related to chemical evolution in the prebiotic environment. On the other hand, tide pools as sites for the origin of life are particularly vulnerable to the sterilizing effects generated by giant impacts. For this reason, although life may have begun in the million-year intervals between impacts, the only forms of life that could survive such events would be chemotrophic thermophiles protected in deep marine environments similar to contemporary hydrothermal vents. If this were true, thermophilic microorganisms would resemble the last common ancestor, in agreement with the observations of molecular phylogeny (165).

The assumption of a microenvironment is useful in designing research approaches related to the subject of this review. Pertinent microenvironments include mineral surfaces with adsorbed films and membrane-encapsulated volumes. Microenvironments are a ready source of concentrated reactants, in that molecular films of organic substances adsorbed to a mineral surface or self-assembled into supramolecular structures such as micelles or bilayers are near the upper limits of concentration. Microenvironments also have the useful feature of bringing together specific kinds of reactants. For instance, anionic solutes will adsorb to cationic mineral surfaces (152) and a membrane-bounded volume will differentiate between solutes based on permeability to lipid bilayers.

Freshwater or Seawater as a Reaction Medium?

Origins-of-life research is typically carried out under sterile laboratory conditions with clean glassware, distilled water, and pure reagents. However, it is likely that the earliest microbial life developed in physically and chemically complex marine environments, either at intertidal zones or perhaps in hydrothermal regions (11, 28). Seawater is a mixture of relatively high-ionic-strength mineral salts, and this fact leads to experimental difficulties that have not yet been addressed. For example, the calcium and magnesium in seawater tend to precipitate a variety of anions, including phosphate itself. In the particular case of simple lipids, seawater is "hard water" and any fatty acids present would quickly aggregate into insoluble curds. Another consideration is that the high osmolarity of seawater (0.45 M NaCl) means that primitive cells would necessarily develop some way to cope with osmotic pressure gradients across their membranes.

Such concerns might be overcome if life began in freshwater, but other difficulties then arise. Even on the present Earth, lakes, rivers, and ponds represent only 1% of the hydrosphere and tend to be relatively short-lived compared to permanent oceans. At the time of the origin of life, true continents had not developed, further limiting the availability of freshwater. Given these considerations, it is unlikely that nutrient solutions with the potential to support complex prebiotic chemistry would have occurred in freshwater environments.

Availability of Prebiotic Phosphate

Westheimer (162) has convincingly argued that the chemical properties of phosphate make it a clear choice for the central role it plays in bioenergetic pathways. Phosphate is a primary limiting nutrient in the present biosphere, and it is interesting to ask if phosphate availability might also have been limiting for the synthetic chemistry that led to prebiotic phosphorylated compounds (56, 132). Phosphorus makes up approximately 0.1% of the Earth's crust, or 2×10^{19} kg. Due to the limited aqueous solubility of calcium phosphate salts at the pH of seawater and the inaccessibility of most of the crust to aqueous solutions, only a small fraction of crustal phosphate is in fact available for biological reactions. We can get some idea of the actual amount from estimates of total phosphate present as sedimentary minerals. Apatite and related minerals (fluoroapatite, carbonate apatite) constitute at least 90% of the available phosphate mineral. One estimate of sedimentary phosphate minerals (expressed as P_2O_5) is 48×10^{12} kg (27), which would produce a concentration of about 0.2 μM if dissolved in the ocean. The ocean presently contains about three times this concentration of phosphate (0.66 μM), suggesting that most of the available phosphate is already in solution, with only minor amounts trapped in minerals and biomass. This leads to the surprising conclusion that phosphate availability probably constrained prebiotic evolution leading to the first life, just as it limits biomass production today. Because micromolar concentrations are unlikely to participate in organic phosphorylation reactions, it follows that concentrating mechanisms must have been available on the early Earth (132).

ORGANIC COMPOUNDS ON THE EARLY EARTH

With our increasing understanding of the early history of the Earth and its relationship to the fossil record and molecular phylogeny, the question of how life began has taken on much broader dimensions. In the past, the problem was confined largely to a chemical approach concerning the monomers common to the primary macromolecules involved in living systems: were amino acids, sugars, and purines/pyrimidines available on the primitive Earth, and, if so, how did they get there? The classic experiments of Miller and Urey (96, 98) showed that impressive yields of amino acids could be obtained when a mixture of reduced gases was exposed to an electrical discharge. The mixture was assumed to be a simulation of the original terrestrial atmosphere, which, by analogy with the outer planets, would have contained hydrogen, methane, ammonia, and water vapor. At sufficiently high energy fluxes, such reducing systems of gases generate hydrogen cyanide and formaldehyde, which in turn react through the Strecker synthesis to produce amino acids.

Cyanide and formaldehyde are now considered to be key reactants in simulations of prebiotic chemical pathways (49, 50, 103). For instance, Oró and Kimball (113) found that purines

such as adenine can be synthesized as pentamers of hydrogen cyanide. Furthermore, the formose reaction was well known to synthesize a variety of sugars from formaldehyde (41). The assumption that organic material readily formed under prebiotic conditions was given additional weight when it was convincingly shown that carbonaceous meteorites contained a remarkable mixture of organic compounds, including amino acids, hydrocarbons, and even traces of purines (29, 83). If such meteorites represent samples of the primitive solar system that underwent synthetic chemical reactions, it was reasonable to assume that similar reactions may have occurred on the Earth's surface. Thus, with organic monomers conceivably available in reasonable concentrations on a global scale, it was not difficult to imagine that self-assembling systems of polymerized macromolecules would at some point attain the properties of the living state.

This optimistic picture began to change in the late 1970s, when it became increasingly clear that the early atmosphere was probably volcanic in origin and composition, composed largely of carbon dioxide and nitrogen rather than the mixture of reducing gases assumed by the Miller-Urey model (68, 77, 156). Carbon dioxide does not support the rich array of synthetic pathways leading to possible monomers, so the question arose again: what was the primary source of organic carbon compounds?

One possibility is that extraterrestrial infall in the form of micrometeorites and comets provided a significant amount of organic carbon. This conjecture was first proposed by Oró (112) and Delsemme (44) and more recently elaborated by Anders (1) and Chyba et al. (23, 24). The total organic carbon added by extraterrestrial infall over $\sim 10^8$ years of late accretion has been estimated to be in the range of 10^{16} to 10^{18} kg. This is less than the total organic carbon stored as oil shales, coal, and other deposits on the Earth (10^{21} kg) but is several orders of magnitude greater than the total organic carbon in the biosphere, estimated to be 6×10^{14} kg. It therefore seems reasonable to conclude that extraterrestrial infall was a significant source of organic carbon in the prebiotic environment.

Most of the cometary and meteoritic infall surviving atmospheric entry and impact would presumably fall into oceans and release its organic content over long time intervals. Mechanisms for releasing organic components from extraterrestrial infall have not been considered in any detail. Recent observations from oil chemistry suggest that hydropyrolysis conditions may provide a useful extraction mechanism (142). In this process, an organic-mineral matrix is simply heated in the presence of water and high pressure to 200 to 350°C for periods up to several days. The hydropyrolytic process would release organic compounds from polymeric structures, thereby markedly increasing the concentrations of small molecules in the prebiotic environment. In one test of this idea, samples of the Murchison carbonaceous meteorite were exposed to hydropyrolytic conditions and the extracted components and the remaining mineral matrix were analyzed by mass spectrometry (92). Gas chromatograms of products isolated from hydropyrolyzed Murchison samples showed an abundant array of organic compounds. Comparison of the carbon content of the mineral matrix by gas chromatography and mass spectral analysis before and after hydropyrolysis showed that over half of the carbon had been released as soluble organics. It is interesting that the extracted compounds were found to be capable of supporting cultures of certain oligotrophic bacteria with carbon, nitrogen, and chemical energy, suggesting that extraterrestrial infall could have provided nutrients for primitive microorganisms.

Hydrothermal conditions would presumably have been relatively common on a highly volcanic prebiotic Earth. It seems reasonable that extraterrestrial infall in the form of micrometeorites and cometary debris would sink in early oceans to regions of volcanic activity, where water temperatures and pressures are conducive to hydropyrolysis. If extraterrestrial delivery followed by hydropyrolytic extraction was a plausible source of organics, it seems likely that such compounds were among the most common components in the prebiotic organic inventory. This would have significant implications for our understanding of the origin of life.

The idea that a significant fraction of the carbon compounds required for the origin of life may have had an extraterrestrial source is startling, but there is no doubt that the planets accreted from a nebular disk of dust and gas around the early Sun, so that in fact all of the primary biogenic elements on the Earth (carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur) had an extraterrestrial origin. The only question is how much chemical processing occurred before the elements were incorporated into primitive forms of life. Either organic compounds were synthesized at the Earth's surface or preexisting extraterrestrial organic compounds were delivered to the Earth's surface by meteoritic and cometary infall. There are arguments for and against either scenario, but for the purposes of this review, we will assume that the earliest forms of life could have used nutrients and energy from either source.

ENERGY SOURCES FOR EARLY FORMS OF LIFE

In considering the sources of energy available in the prebiotic environment, we can first ask what global-scale energy fluxes could have driven the chemical evolution leading to increasingly complex organic molecules. Figure 3A shows estimates of such energy sources, and it is clear that light energy is the most abundant by 2 to 3 orders of magnitude. However, the efficiency by which energy sources are used for chemical synthesis must also be considered (145), so that other forms of energy, even though less abundant than light, can be significant in driving synthetic reactions. These global energy inputs have been incorporated into a variety of schemes that would lead to the synthesis of simple organic compounds, and estimated accumulations are shown in Fig. 3B (24). An important example of a primary photochemical pathway is the atmospheric synthesis of formaldehyde (121), which was calculated to be produced at a rate of 10^{11} mol per year. To give a perspective on this value, if we assume that synthesis proceeded for 10^7 years and that no degradative reactions occurred, this rate would result in the accumulation of 10^{-3} M formaldehyde in the ocean, a concentration sufficient to initiate secondary reactions leading to more complex molecules.

We will next consider energy sources available for prebiological systems at the local scale and in microenvironments. In concept, capture of these energy sources can be understood in terms of either contemporary metabolic pathways or cell structural components. The conceptual link to metabolism is to assume that a network of chemical reactions developed primarily as solution chemistry and later evolved into the synthetic pathways associated with the living state (42, 101, 152, 153, 159). This proposal has a potentially testable prediction, in that remnants of the network might be found in the metabolic pathways of extant organisms.

The idea that energy could be captured by macromolecular structures rather than chemical reactions in solution can be traced back to Oparin's proposal that colloidal gels (coacervates) happened to incorporate chemical reactions that allowed them to grow and reproduce (107). A more contemporary version is that membrane compartments were available on

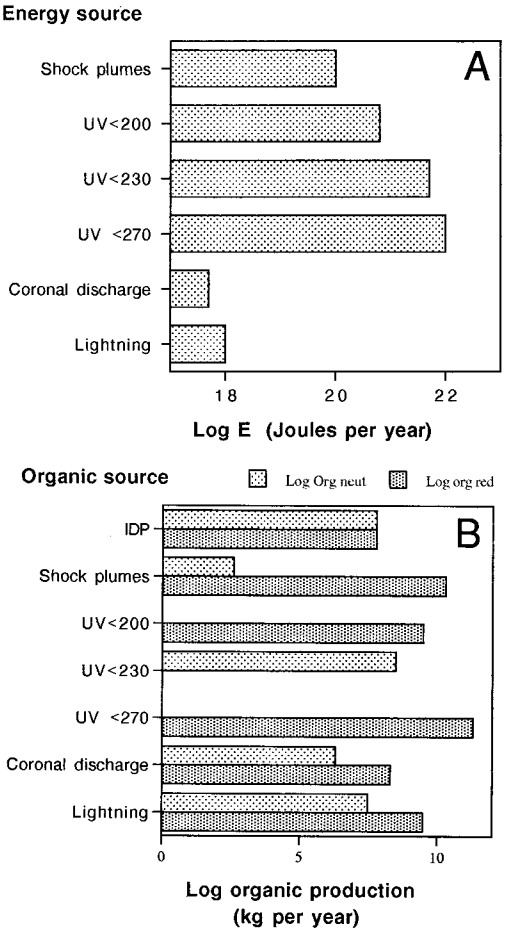

FIG. 3. Estimates of energy and organic synthesis on the early Earth. (A) Global energy inputs. Energy sources capable of driving organic synthesis include UV light, electrical discharge, and atmospheric shock produced by extraterrestrial infall. Of these, photochemical synthesis is likely to have been a primary source of organic compounds, an important example being formaldehyde (121). (B) Organic synthesis driven by energy inputs on a global scale. The values have been corrected for the efficiency with which various forms of energy can drive synthetic reactions (145), and results for both reduced atmospheres and neutral atmospheres are shown. UV light and, to a lesser extent, lightning, are responsible for the major fraction of organic synthesis. The estimated organic input from interplanetary dust particles (IDP) is also shown. For comparison, the total carbon now present in the biosphere is 6×10^{14} kg. Data from reference 24.

the early Earth, self-assembling from amphiphilic compounds in the environment and later providing a suitable microenvironment for energy-dependent life processes (19, 80, 101, 102). It can even be argued that such membranes could take on the process of reproduction, quite apart from any genetic apparatus (89, 102). If we assume that such membranes were sufficiently impermeable to ions to develop electrochemical ion gradients, it is possible that chemiosmotic energy sources were available even to the earliest forms of life (80, 100). If pigments were available to capture light energy, such systems would represent an autotrophic origin of life.

A third possibility is that the earliest forms of life had the ability to capture redox energy, for instance by electron transport from hydrogen or hydrogen sulfide to electron acceptors such as iron in solution or present as a mineral surface (18, 153). This would provide an energy source for autotrophic life existing in a local-scale environment such as submarine hydrothermal regions.

In the discussion to follow, I discuss specific forms of energy that were available to early forms of life and then critically assess whether a given source of energy could plausibly be incorporated into a primitive microorganism to drive metabolic processes. The primary forms are easily enumerated for local and microenvironments: heat energy, chemical energy (including oxidation and reduction reactions), and light energy. I then consider possible sources of chemiosmotic energy, which will necessarily address the question whether primitive membrane structures were plausibly available on the early Earth.

Heat Energy

Heating and drying is one of the more robust ways to drive the condensation reactions required to produce polymers of simple organic molecules. Dry heat can activate dehydration reactions in which water is lost and a variety of covalent bonds are produced. The chemical potential is provided by anhydrous conditions, so that condensation reactions become favorable as water leaves the reactants. In early work, Fox and Harada (54) demonstrated that simple heating of amino acid mixtures to temperatures in the range of 160°C produced polymeric substances that they termed proteinoids. Under some conditions, the polymers could be induced to form spherical structures referred to as protocells, and Rohlfing (126) has shown that, given time, similar polymers can form at lower temperatures as well. Fox went on to test proteinoids and protocells for suggestive chemical properties, including catalytic function and light transduction, but for the most part this approach has been abandoned. Even though heating and drying remains an attractive way to introduce polymers of amino acids into the prebiotic environment, it is not easy to imagine how proteinoids could evolve into true replicating molecular systems now associated with nucleic acids. Furthermore, Fox and Dose (53) used gram quantities of mixtures of pure amino acids in their simulations. It has not been convincingly demonstrated that very dilute solutions of amino acids mixed with other organic solutes can assemble into polymeric structures in simulated early Earth environments.

Heating and drying has also been used to drive polymerization of oligonucleotides. For example, Usher (148) has discussed the ubiquitous nature of heating and drying as a condensation mechanism and has developed a model based on actual temperature ranges of the contemporary Earth. It was possible to demonstrate experimentally that heating could drive phosphodiester bond formation in which a 12-base oligonucleotide was produced from 6-base oligonucleotides in the presence of a complementary template (150). Usher and McHale (149) also showed that the 3′-5′ bonds characteristic of modern nucleic acids are thermodynamically more stable than 2′-5′ bonds under such conditions. There have been few published attempts to carry this work further, probably because of the success of other experimental approaches to be discussed below.

Beyond driving condensation reactions such as those described above, anhydrous heat is not a useful energy source because specific reactions are not readily driven. Instead, formation of polymeric tars is a more common outcome. Heat can also degrade organic substances through pyrolysis, and volcanic activity has been proposed as a significant pathway that continuously removes organic compounds from the early environment. For example, using reasonable assumptions, one may calculate that the equivalent of the entire global ocean volume passes through deep-sea hydrothermal vents every 10 million years (5). The highest temperatures associated with some hydrothermal vents (>300°C) would presumably degrade

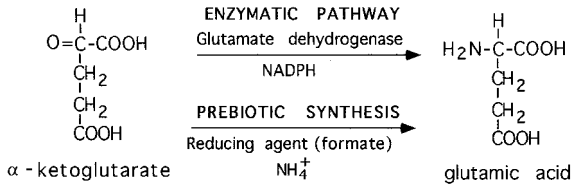

FIG. 4. Proposed synthesis of an amino acid by reductive amination. The major metabolic pathways of contemporary organisms presumably evolved from simpler reactions in the prebiotic environment (see reference 97 for a review). For example, glutamate dehydrogenase catalyzes the synthesis of glutamate from α-ketoglutarate and ammonia. The same synthesis occurs spontaneously in solution in the presence of a reductant such as formate (99).

all but the most robust organic solutes. On the other hand, Shock et al. (137–139) have argued that a variety of organic compounds, including amino acids, could be synthesized at lower temperature ranges, which are more commonly associated with hydrothermal conditions. Resolution of this controversy awaits further experimental analysis (141).

Chemical Energy

Abundant sources of chemical energy are highly plausible components of the prebiotic environment. Chemical energy present in nutrients is released in contemporary cells through metabolism, and the argument that early life was heterotrophic springs from this line of thinking. For instance, Morowitz (101) proposed that metabolism could develop from specific reactions that were spontaneous on the early Earth but have now become entrenched in a defined series of enzyme-catalyzed reactions. One example is the manner in which nitrogen is brought into such pathways. Morowitz (101) noted that in contemporary metabolism, this is done by reductive amination catalyzed by glutamate dehydrogenase, with NADPH as a source of reducing power.

Morowitz et al. (99) tested this concept by determining whether there was a simple nonenzymatic parallel to this reaction, and in fact they were able to demonstrate the synthesis of glutamate from α-glutaric acid in the presence of ammonium salt with formate as the reducing agent (Fig. 4). However, metabolic pathways are now catalyzed by a series of enzymes that not only speed up the reactions by enormous factors but also regulate the flow of metabolites through the living system. This fact is a critical test of all proposed sources of energy on the early Earth. Can a given energy source plausibly drive specific kinds of reactions in a complex prebiotic environment containing mixtures of dissolved organic substances? Or could life processes have been initiated only in a specialized microenvironment that reduces the complexity and thereby constrains the potential reaction pathways? I now review several specific sources of energy that have been proposed for primitive forms of life and then discuss how they could interact with possible systems of encapsulated reactions associated with the living state.

Condensing agents. One alternative to heat-driven condensation reactions is the possibility that chemical condensing agents were present in the prebiotic environment (69). Oró and coworkers have carried out extensive investigations of such reaction systems and have demonstrated that compounds such as cyanamide have useful properties in this regard (104, 115). In one remarkable series of studies, the entire gamut of lipids was synthesized with the help of condensing agents, including molecules as complex as phosphatidylcholine (46, 47, 124). Although it is plausible that a variety of condensing agents were available on the prebiotic Earth, such agents resemble anhydrous heating in being relatively nonspecific. Condensing agents can drive specific reactions in a test tube environment with pure materials, but it is not immediately apparent how such agents could achieve specificity in a mixture of organic compounds on the primitive Earth. Another concern is that it is not easy to come up with continuing sources of condensing agents to drive the polymerization reactions associated with early biosynthetic processes.

Pyrophosphate bond energy. Baltscheffsky (6–8) has argued that pyrophosphate bond energy is an attractive energy source for earlier microorganisms. First, pyrophosphate bonds readily form when inorganic phosphate is dried and heated to temperatures in the range of 200°C, similar to what one might find under volcanic conditions (166). At least one pyrophosphate mineral has been described, supporting the possibility that pyrophosphate was available in the early Earth environment (127). Another favorable argument is the ubiquitous use of pyrophosphate bond energy in contemporary organisms. There must have been a point at which pyrophosphate chemistry became linked with the life process, and it seems likely that the earliest microorganisms would have had metabolic pathways involving pyrophosphate bonds as an energy source.

The notion of pyrophosphate as a plausible energy source for early cells also has significant limitations. As noted above, phosphate is a trace nutrient in the biosphere, and geological conditions that could have provided a concentrated source of phosphate have not been established. However, even given sufficient phosphate in a local environment and assuming that it was in some fashion continuously formed into pyrophosphate to balance the hydrolytic breakdown that would otherwise remove it, how could the first cellular forms of life have taken advantage of pyrophosphate bond energy? Most phosphate chemistry in cellular metabolism is based on enzyme-catalyzed group transfer or enzyme-catalyzed hydrolysis. In the absence of catalyzed reaction pathways, there is no obvious way that such reactions could be incorporated into the primitive forms of life we are discussing here.

Glyceraldehyde. Weber and Hsu (159, 161) have proposed that glyceraldehyde and glyceric acid esters could have been central molecules in the chemical evolution leading to metabolism. One such reaction sequence is shown in Fig. 5, and several attractive features of the glyceraldehyde model immediately become apparent. First, glyceraldehyde is a relatively simple molecule, which could be synthesized from formaldehyde produced in atmospheric reactions (121), thus providing a continuous source. Once synthesized, glyceraldehyde can be incorporated into a variety of secondary reactions as shown in Fig. 5, thereby generating other useful reactants such as glycerol and energy-rich glyceroyl thioesters (158). Weber has argued that such a pathway could plausibly evolve into modern metabolism.

A limitation of this argument is that there is no obvious way to link glyceraldehyde reactions to the primary reactions of life, that is, catalysis, polymer synthesis, and replication of macromolecules. Weber does point out that glyceraldehyde can itself undergo polymerization into polyglyceric acid (160) and that the polymers in principle could act as catalytic and genetic molecules. However, no experimental evidence is available to test the plausibility of this point.

Pyrite as a source of chemical energy and molecular order. Perhaps the most elaborate scheme for a primitive energy source and associated primordial metabolic pathways is that of Wächtershäuser (152, 153), who proposed that the first metabolic pathways took place in films of simple organic compounds coating pyrite mineral surfaces. In this proposal, such

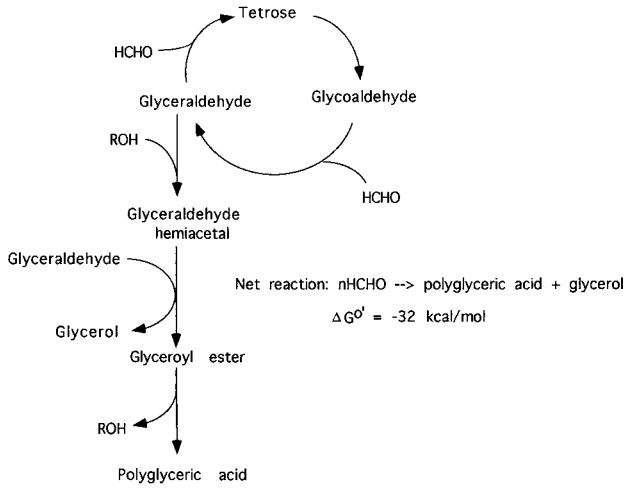

FIG. 5. Weber (159) has demonstrated that glyceraldehyde can participate in a variety of reactions pertinent to early biochemical and bioenergetic processes. For example, the synthesis of polyglyceric acid from formaldehyde shown in this diagram has a highly favorable free energy. Similar pathways can synthesize compounds such as glycerol, alcohols, tetroses, and glyceraldehyde itself, now a central molecule in glycolysis.

films undergo a variety of energetically favorable reactions, with examples including polymerization reactions, lipid chain synthesis from isoprene derivatives, and fixation of carbon dioxide to formic acid. One family of the polymeric materials is referred to as tribonucleic acids, which have the potential to act as a backbone structure of a primitive genetic material.

Pyrite, in particular, has a number of features that make it pertinent to the origin of metabolic pathways. For instance, Wächtershäuser noted that pyrite has a cationic surface, so that anionic organic compounds would presumably be absorbed to its surface through favorable electrostatic interactions. Such films could then undergo a kind of surface metabolism driven by thermodynamically favorable reactions. For instance, Wächtershäuser speculated that glyceraldehyde phosphate and dihydroxyacetone phosphate could polymerize on pyrite surfaces, as shown in Fig. 6A. The free energies of the individual step and overall sequence are highly favorable to drive the forward reactions.

Another example, perhaps the most intriguing, is that pyrite formation can serve as a source of reducing power (153). One such reaction is shown in Fig. 6B, in which ferrous iron reacts with hydrogen sulfide to produce pyrite and free electrons. It should be noted that ferrous iron maintains its valence state in pyrite, so that the electrons are derived from the hydrogen sulfide. The reaction is energetically highly favorable because pyrite is removed from the reaction as a virtually insoluble precipitate. Wächtershäuser went on to propose that pyrite formation could be coupled to carbon dioxide reduction in a thermodynamically favorable reaction, as shown in steps 2 and 3 in Fig. 6B. A variety of other reactions were also proposed, all of which have various degrees of continuity with contemporary metabolism. For instance, it is possible that at some point, films of lipid-like compounds synthesized on the pyrite surface could self-assemble into bilayer structures. The resulting membranes would peel away to produce cellular life forms that maintain the metabolic pathways originally developed on the pyrite surface.

Although this proposal has a number of ingenious features, it is also open to critical commentary. DeDuve and Miller (43) have discussed a number of shortcomings. One is that the proposed reactions are entirely hypothetical and have little experimental support. They also pointed out that the thermodynamic aspects are improbable. For instance, only two forms of free energy are proposed to drive the reactions: sulfur oxidation and bonding of anions to charged surfaces. However, no coupling reactions are proposed to link sulfur oxidation to the other reactions. Furthermore, if ionic bonding of substrates to pyrite surfaces is an absolute requirement, the proposed surface reactions may undergo a round of polymerization but eventually will end up as a strongly bound coat of polymer, an evolutionary dead end.

Some experimental tests of the concepts have been carried out, with limited success (67, 76, 79a). These experiments have demonstrated modest levels of chemical reactivity and reducing power, but it is fair to say that none of the primary reaction pathways proposed by Wächtershäuser have yet been established in the laboratory.

Activated monomers. Another form of prebiotic chemical energy is in the form of activated monomers. In this scenario, it is supposed that some source of relatively high-energy biomolecules was available, so that polymerization reactions became energetically favorable. DeDuve (42) summarized this as follows: "The pathway to life must have been downhill all the way, with at most a few rare humps that could be negotiated with the help of the acquired momentum. One would expect such a roadway to be readily visible. Yet, so far, like some artfully hidden jungle trail, it has eluded every search, despite extensive experimentation and much imaginative theorization and speculation."

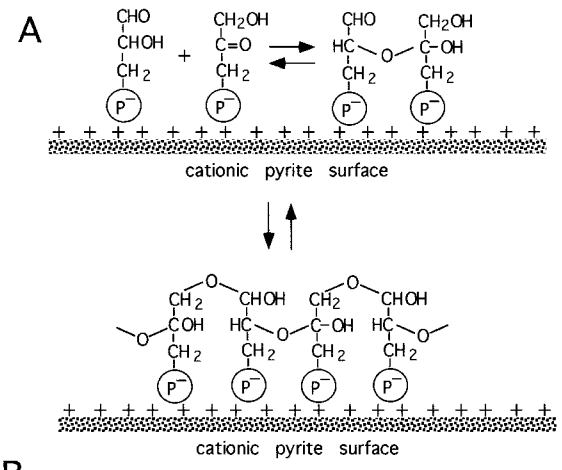

FIG. 6. Examples of possible reactions involving pyrite (152). (A) Assuming that pyrite has a cationic surface, a variety of anionic reactants would be adsorbed to the surface and potentially undergo reactions. The example shown here is the adsorption of glyceraldehyde-3-phosphate to the surface, followed by polymerization. (B) Participation of pyrite in a reaction can drive an otherwise energetically unfavorable reaction. For instance, reduction of carbon dioxide by hydrogen has a positive Gibbs free energy (reaction 1). However, pyrite formation from ferrous iron and hydrogen sulfide is energetically favorable and can make reducing power available (reaction 2). If carbon dioxide reduction is linked to this reaction (reactions 3 and 4), the synthesis of formic acid (a reduced form of carbon dioxide) is energetically favorable.

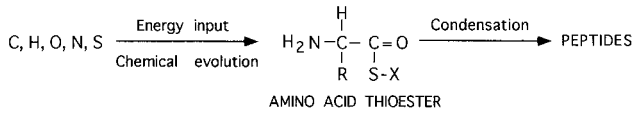

FIG. 7. It has been proposed that prebiotic synthesis of activated monomers could occur on the early Earth (42). Important examples of such compounds are thioesters of amino acids shown here, which readily undergo condensation reactions to form peptide bonds.

Arguing from the role in contemporary cells of thioesters, such as coenzyme A, and from Lipmann's discovery that peptides such as gramicidin are synthesized from thioester intermediates in the absence of ribosomes and related translation mechanisms, DeDuve suggested that possible activated monomers are the thioesters of amino acids (Fig. 7). Peptide synthesis from such esters occurs spontaneously (165), and a pathway to thioesters has been demonstrated by Miller and Schlesinger (95). Weber (158) has also proposed a reaction for thioester synthesis. However, no obvious continuous source of thioesters has been established. Furthermore, even if thioesters were present, there is no catalytic template available to guide them into specific patterns, so that the resulting peptides would be a random assortment of sequences, with no clear ability to self-replicate.

The imidoesters of amino acids and nucleotides are another version of activated intermediates. This reaction was pioneered by Lohrmann and Orgel (87, 88, 111) and has been extensively investigated over the past two decades (73–75, 133). In a typical reaction, chemically activated nucleotides are allowed to interact with a template that has favorable base-pairing possibilities. Examples of chemically activated monomers include imidazole esters of mononucleotides, with the imidazole acting as a leaving group. The monomers line up along the template, and the energy is released as phosphodiester bonds are synthesized.

This system has provided significant insight into what might be required of a true self-replicating system of molecules. For instance, it was quickly found that 2′-5′ diester bonds tend to form, rather than the 3′-5′ bonds characteristic of RNA and DNA. Later work (70) showed that 3′-5′ bond formation could be promoted by the inclusion of metal ions such as zinc or lead in the reaction medium. Another highly significant observation was that the reaction was markedly inhibited if a racemic mixture of activated nucleotides, rather than pure D-ribonucleotides, was used (75). The sensitivity to mixed stereoisomers offers a strong clue to why chirality developed in the origin and evolution of living systems. Perhaps the most significant limitation is that activated purine nucleotides readily polymerize on a pyrimidine template, but there has been no experimental success in closing the cycle by demonstrating that pyrimidine nucleotides can polymerize on a purine template (72, 110). In other words, the template-dependent polymerization as it stands is a one-step reaction and could not be incorporated into a full system capable of continuous template-directed growth.

Light Energy

Although the energy sources described above provide useful insights into the chemical requirements of nonenzymatic metabolism, we come quickly to a very difficult question: what were plausible examples of prebiotic activating mechanisms that would produce a constant supply of chemical energy? After chemical energy, the two most common energy sources used by contemporary prokaryotes are light energy and ionic potentials, commonly referred to as chemiosmotic energy. However, if either is to be used by a primitive microorganism, it implies that specific chemical and physical structures were available in the environment. That is, light energy transduction requires a pigment to capture the light energy, while chemiosmotic energy requires a membrane with sufficient capacitance to maintain electrochemical gradients for a useful period. I first discuss whether plausible pigment systems might have been available.

Light energy was presumably the most abundant source of energy on the prebiotic Earth, just as it is today. However, to capture the energy, the light must first be absorbed by some photochemical process and then transduced into other usable forms of energy rather than being degraded into heat or fluorescence. It follows that there must have been an original photosynthetic process having evolutionary continuity with contemporary photosynthesis. The nature of the first pigment systems remains an open question. One approach is to look at early pigments not as products of biosynthetic pathways but, rather, as nutrient constituents of the organic mixture available to early forms of life, in the same sense that contemporary cells depend on environmental nutrients such as essential fatty acids and essential amino acids and vitamins. Are there any compounds that might plausibly fulfill this role? A few that have been proposed to occur on the early Earth include ferrous iron and complex ions such as ferrocyanide (2, 16), porphyrins (94), proteinoids (53), and polycyclic aromatic hydrocarbons (PAHs) (33).

Before going on, I discuss the tasks that a primitive pigment could usefully perform. Contemporary photosynthetic pigment systems use light energy to transfer electrons from water to carbon dioxide, thereby making chemical energy available for the biosphere. As the electrons travel through a complex electron transfer chain, energy is conserved in the form of electrochemical proton potentials, pyrophosphate bond energy, and reducing power. This highly evolved reaction sequence is only now being understood at the molecular level, and it turns out to have very precise structural and chemical requirements. It is unlikely that such a system could spring full-blown from the prebiotic mix. Therefore, I will break down the sequence into individual steps and ask whether plausible prebiotic assemblies of organic compounds might be capable of a given step. A list of the most general steps would include (i) transfer of electrons from a pigment donor molecule to an acceptor molecule; (ii) proton uptake and release, coupled to production of proton gradients across membranes; (iii) hydrolysis of water to hydrogen and oxygen; (iv) generation of a voltage across a membrane; and (v) pyrophosphate bond formation.

Generation of pH gradients. I first consider reactions that could be used by a developing protocellular system to produce proton gradients across membranes. One such reaction would involve a light-absorbing pigment either captured by a vesicle or present in the lipid environment of an early membrane. The pigment would in some manner undergo a relatively simple photochemical reaction that releases or takes up protons, resulting in a proton gradient that captures a portion of the original light energy. At some point in early evolution, the gradient energy would be coupled to other useful processes such as nutrient transport or chemical bond synthesis.

The first model I consider is an encapsulated soluble system that incorporates the photochemical properties of iron. Complex ions of iron have been proposed as plausible components of early oceans (2), and their chemistry has been the subject of numerous investigations. Ferrocyanide has the interesting property that in solution it can absorb near-UV light and release cyanide ions (36). Because cyanide is a weak acid with

a pK_a of 9.2, at neutral pH ranges the cyanide ion can associate with protons to produce a marked increase in the pH of its solution. For example, a few seconds after illumination begins, the pH of unbuffered 1.0 mM potassium ferrocyanide increases from 6 to 9. If the ferrocyanide is encapsulated in liposomes, substantial pH gradients of 2 to 3 units can be generated across the membrane upon illumination (36). These results show how unexpectedly simple it may be to generate pH gradients. Although this photochemical reaction of encapsulated ferrocyanide has no obvious continuity with a pathway that might have evolved into primitive photosynthesis, the system does provide a model with which to investigate the requirements for early chemiosmotic processes.

PAHs are a second potential pigment system. PAH derivatives in the form of kerogen-like polymer represent over 90% of the organic material of carbonaceous chondrites (29). They are also likely components of micrometeorites and comets, and so it is reasonable to think that PAH would be available in the early Earth environment. Many PAH derivatives absorb light in the near-UV and blue region and are therefore likely to be photochemically reactive. Because they are relatively nonpolar, PAH molecules would tend to partition into membranes, and photochemical reactions could then transduce light energy directly into chemiosmotic potential.

The literature has abundant examples of photochemical reactions pertinent to the expectations of a primitive photosynthetic system. For instance, if pyrene is prepared in phospholipid bilayers, it can release a hydrated electron upon illumination and reduce benzophenone (48). This reaction represents a photochemical pathway with clear relevance to light energy capture by a primitive pigment system. That is, a photon generates an excited state of a membrane-bound pigment, an electron is released, and an acceptor molecule is reduced. In another relevant PAH reaction, when 1-naphthol is illuminated and shifts to an excited state, its pK shifts dramatically from 9 to 0.5, so that a proton is transiently released (64). A third experimental system was established by Warman et al. (157), who synthesized a molecule with a dimethoxynaphthalene group attached to a hydrocarbon chain with two cyanide groups at the other end. Upon illumination, the excited-state molecule becomes a "giant dipole" with properties similar to those occurring in the initial steps of photosynthesis.

Several PAH model systems have been explored in an attempt to trap the released protons across a membrane in the form of chemiosmotic proton gradients. One such model is a mixture of a long-chain alkane with dissolved PAHs, prepared as microemulsions in salt solutions. When such dispersions are illuminated under anaerobic conditions, marked acid pH shifts are readily observed. For instance, 2-ethylanthracene dispersed in hexadecane as microemulsions produced pH shifts up to 3 units (151), equivalent to hydrogen ion concentrations in the millimolar range. Given that PAH derivatives readily produce protons upon illumination, it should be possible to generate pH gradients across lipid bilayers. In one experimental protocol, PAH derivatives were included in lipid bilayer membranes (liposomes) prepared from phospholipids (33). Upon illumination, substantial pH gradients were established across the membranes, acidic inside. This is not a true pump, since the protons produced by the PAHs simply accumulate inside the vesicles, which have a small volume relative to the large external volume. Nonetheless, this experiment once again demonstrates that, given a membrane-enclosed vesicle and a source of free energy, pH gradients are not only likely but almost inevitable.

Porphyrins as early pigments. As long ago as 1957, Granick (59) proposed that porphyrin derivatives were likely precursors of photosynthetic pigment systems. In more recent investigations, it has been demonstrated that porphyrins can be incorporated into lipid bilayers and experience light-dependent excited states that produce voltage and ionic currents across the membrane. I will describe two such experimental model systems here.

Seta et al. (134) reasoned that a molecule composed of three functional moieties—a pigment, an electron donor, and an electron acceptor—might be able to use light energy to separate charge across a bilayer in which it was embedded. A "triad" was therefore synthesized which contained a porphyrin head group with attached quinone, the porphyrin in turn being linked to a carotene chain that provided a chain of conjugated bonds to deliver electric charge across the bilayer. The triad was inserted into a planar lipid bilayer and illuminated in the presence of a redox couple, ascorbic acid on one side of the bilayer and ferricyanide on the other. Under these conditions, a transmembrane photocurrent with a peak of 200 pA was immediately produced upon illumination. This is best explained by a mechanism in which individual triads form an excited-state species, $C^{2+} - P - Q^{-}$, within 100 ps, which relaxes by picking up an electron from the ascorbate and delivering it to the ferricyanide. Each triad performs this task multiple times, resulting in measurable photocurrents even against opposing voltages of 100 mV.

A second porphyrin-based system was described by Sun and Mauzerall (146). In this model, magnesium octaethyl porphyrin was partitioned into a planar lipid membrane composed of diphytanoyl phosphatidylcholine. Anthroquinone-2-sulfonate was added to one side of the membrane as an electron acceptor. Chlorosubstituted tetraphenylborate was added to both sides of the membrane to act as a mobile charge carrier. When the bilayer was illuminated by a single laser pulse, a photovoltage of 2 mV was produced in a few microseconds, which then decayed over periods of microseconds to milliseconds. Under continuous illumination, a relatively large voltage of 10 mV was generated with resulting currents of 170 pA. These results were explained by formation of a magnesium porphyrin cation (P^{+}) in the bilayer, which draws the borate anions into the bilayer and allows them to deliver an electron to anthroquinone-2-sulfonate on the other side and then return for another cycle. The overall result is a kind of pumping cycle, with efficiencies around 30%.

Ionic Potentials across Membranes

Several investigators have suggested that early cells could have developed ionic potentials from which energy in the form of a chemiosmotic potential could be derived (37, 80–82, 100, 101). Koch and Schmidt (81, 82) have proposed a fairly explicit hypothetical mechanism by which cells would generate a chemiosmotic potential and link it to the synthesis of high-energy phosphate bonds (Fig. 8). In this scheme, hydrogen is used as a source of electrons, which are donated to an internal acceptor so that charge is separated across the bilayer. In a second reaction, external inorganic phosphate forms a bond with a compound analogous to creatinine, which is linked to a hydrocarbon moiety that makes it membrane soluble. The phosphate then picks up protons so that charges on the phosphate are neutralized, and the positively charged phosphorylated compound is actively transported down the electrochemical gradient to the cell interior. At this point, the phosphate is transferred to an acceptor and the neutral molecule diffuses back across the bilayer to begin a new cycle. The phosphorylated acceptor on the interior now serves as a source of high-

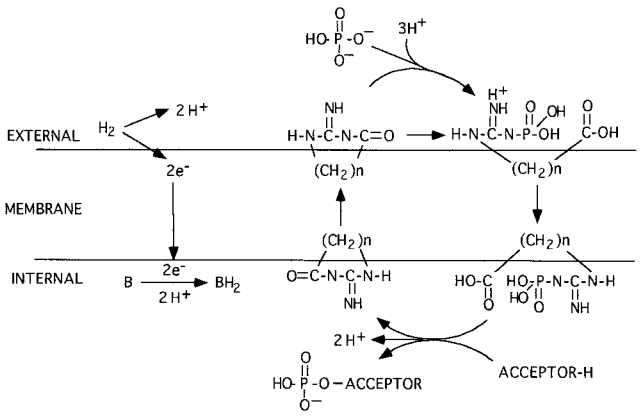

FIG. 8. Koch (81, 82) has proposed reaction pathways that could generate a chemiosmotic potential across primitive cell membranes and be coupled to the synthesis of a high-energy phosphorylated compound (see the text for details).

energy phosphate to drive metabolic reactions and other energy-requiring processes.

This energy conservation pathway has a plausible energy source in the form of molecular hydrogen, which could be made available from a number of geochemical sources. The other components are somewhat less plausible in terms of what we understand about prebiotic chemistry, but it should not be difficult to synthesize compounds similar to those proposed for the phosphate donor and acceptor molecules and determine whether they can function in the manner proposed here. It is also very straightforward to produce electrochemical proton gradients that would drive the reaction. For instance, liposome systems in which ascorbate delivers electrons to encapsulated ferricyanide via a membrane permeable electron carrier such as phenazine methosulfate have been described (40). Under these conditions, pH gradients in the range of 3 to 4 units are generated across the liposome membrane.

Capture of Free Energy by Primitive Cells

To conclude this section, I apply a conceptual test to the energy sources that have been proposed for primitive life. The test is simple: how plausible is it that a given source of energy could be incorporated into a cellular microenvironment? Obviously, all of the proposed energies will drive reactions under laboratory conditions with pure compounds, but energy sources on the primitive Earth were likely to be dispersed unless they could be captured by a membrane-bounded volume. Therefore, an ideal source of energy is able to be continuously renewed and to drive an encapsulated reaction by donating its energy to one or more steps of an early metabolic pathway. In the absence of these requirements, a given system of reactants will quickly approach equilibrium, as in the thought experiment described earlier.

Perhaps the least useful forms of energy are those involving ions that will have difficulty crossing a membrane. It follows that ions such as pyrophosphate, ferrocyanide, thioesters of amino acids, and activated nucleotides would be less plausible energy sources. By this test, minerals like pyrite are poor energy sources as well. To use a redox couple based on pyrite, for instance, a cell would necessarily encapsulate mineral crystals, which would seem to be an unlikely prospect.

More probable energy sources incorporate small, neutral molecules. For instance, pathways such as those proposed by Weber (159) are reasonable from this perspective, in that glyceraldehyde would readily permeate a membrane-enclosed space to serve as a primary energy source. Pathways based on highly permeable formaldehyde and hydrogen cyanide would also function well, and it would be interesting to see whether an intravesicular Strecker synthesis could be established in a liposome system. Also highly plausible would be redox systems based on hydrogen or hydrogen sulfide. However, if the reducing potential of a gas like hydrogen is to be tapped, it is also necessary to incorporate an electron acceptor to complete the redox couple. It may be significant that most acceptors (with the exception of molecular oxygen) are highly polar or ionic species that would be easily encapsulated by a primitive cell.

Probably the most robust energy source would be a membrane-bound pigment system that could develop a chemiosmotic potential across the membrane. This could be driven either by a light-dependent electron transport pathway that develops a membrane potential or by a proton pump analogous to bacteriorhodopsin. PAH derivatives are an interesting possibility in this regard. They were likely to have been abundant on the early Earth and are readily incorporated into bilayers. However, they lack obvious continuity with contemporary photosynthesis. Porphyrins clearly have evolutionary continuity, but no robust synthetic pathway to porphyrins has been established for the prebiotic environment. Finally, even if a chemiosmotic potential could be established by a pigment system, what mechanism would couple it to the generation of high-energy bonds? This difficult question cannot yet be answered, nor are there convincing experimental approaches.

BILAYER BARRIERS AND EARLY BIOENERGETICS

The last section of this review will discuss the possible presence of membranous compartments on the early Earth. If light energy or chemiosmotic energy was used by the earliest forms of life, membranes are an absolute requirement. The following questions will be addressed. (i) Are there plausible sources of amphiphilic molecules in the prebiotic environment? (ii) If so, is there a simple mechanism by which catalytic and replicating macromolecules could be encapsulated in the compartments? (iii) Last, which permeability properties would be required for generation of a chemiosmotic potential that would also accommodate nutrient transport across the membrane?

The first suggestion that membranes played a role in the origin of life was in Haldane's prescient note in *The Rationalist Annual* (60). Haldane wrote that "The cell consists of numerous half-living chemical molecules suspended in water and enclosed in an oily film. When the whole sea was a vast chemical laboratory the conditions for the formation of such films must have been relatively favourable. . ." Goldacre (58) proposed in 1958 that the first membranes could have been produced by wave action disturbing films of lipid-like surfactants. Following the development of the liposome concept, Bangham suggested that liposome-like vesicles were likely precursors of primitive cellular life (9). Fleischaker (52) has argued that the simplest form of life is a minimal protocell, so that the quality of a membrane boundary should be included in the definition of the living state.

How plausible is the conjecture that self-assembled bilayer membranes were present in the prebiotic environment? And if bilayers were involved in the evolutionary process leading to the first cellular forms of life, what were their permeability properties? It is a reasonable assumption that the first cells would lack the highly efficient membrane transport systems characteristic of contemporary cell membranes, which means that inward nutrient transport would necessarily be accomplished by passive diffusion. Could transmembrane diffusional

processes be fast enough to keep up with the demands of a primitive metabolism? Also, if early membranes were sufficiently permeable that ionic nutrients such as amino acids had access to encapsulated catalysts, how could chemiosmotic potentials be maintained? There is no simple answer, of course, but recent experimental results at least provide a perspective. I begin by outlining the basic physical properties of molecules that can self-assemble into stable bilayers and then discuss the possibility that such molecules were plausibly available in the prebiotic environment.

Bilayers Assemble from a Variety of Amphiphilic Compounds

Although contemporary cell membranes incorporate phospholipids as the primary component of lipid bilayers, it is not necessary to think that complex lipids were required for early cellular life (38). In fact, simpler amphiphilic molecules can also assemble into bilayer membranes (123). These include single-chain amphiphiles such as soap molecules, glycerol monooleate, oxidized cholesterol, and even detergents like dodecyl sulfate mixed with dodecyl alcohol (61). It seems likely that primitive cells incorporated lipid-like molecules from the environment almost as a nutrient, rather than undertaking the much more complex task of synthesizing complex lipids de novo.

Bilayer Permeability Strongly Depends on the Chain Length of the Component Amphiphilic Molecules

Lipid bilayers of contemporary cell membranes present a significant permeability barrier that is necessary for normal cell functions, particularly those related to the bioenergetics of ion transport and chemiosmotic ATP synthesis. This leads to a conundrum when we try to imagine how early forms of cellular life could have functioned in the absence of highly evolved transport enzymes that translocate ionic nutrients and metabolites across the bilayer barrier. As discussed below, bilayers composed of lipids with shorter hydrocarbon chains are more permeable by several orders of magnitude. This level of permeability is sufficient to encapsulate large molecules such as proteins and polynucleotides yet still allow the external substrate to reach an encapsulated enzyme. It follows that early cell membranes could have been composed of shorter-chain lipids that provided access to nutrients for macromolecules undergoing growth and replication in an encapsulated microenvironment.

Macromolecules Can Be Encapsulated in Bilayer Vesicles under Simulated Prebiotic Conditions

A third conceptual problem has been to imagine how lipid bilayers could capture macromolecules in the first place, given that the bilayer must present a nearly impenetrable barrier if the macromolecules are to be maintained within the membrane-bounded volume. It is possible to demonstrate experimentally that lipids mixed with macromolecules such as proteins or nucleic acids can undergo drying and wetting cycles that simulate the tide pool environment. Under these conditions, the macromolecules are readily captured in membrane-bounded vesicles.

Lipid Bilayers Grow by Addition of Amphiphilic Compounds Present in the Bulk Phase Medium

Given that a primitive cell will be able to replicate its encapsulated macromolecular components, it will then be necessary for its boundary membrane to increase in area to accommodate the internal growth. I discuss recent experimental results from liposome model systems that demonstrate a form of growth.

PHYSICAL PROPERTIES OF AMPHIPHILES AND BILAYERS

All bilayer-forming molecules are amphiphiles, with a hydrophilic "head" and a hydrophobic "tail" on the same molecule. Although we tend to think of membrane lipids in terms of phospholipids, a variety of other amphiphiles, such as sphingolipids, glycolipids, cerebrosides, and sterols, can be incorporated in membranes. Archaebacterial lipids incorporate ether bonds instead of esters, and some have long hydrocarbon chains that span the membrane. Perhaps the most remarkable membrane lipid components are those of certain algal cells, which are composed entirely of a mixture of single-chain fatty acids, sterols, and chlorosulfolipids.

Chain length is the primary factor that determines whether a given amphiphile can self-assemble into stable membranes at ordinary temperatures. Earlier work (62) showed that single-chain amphiphiles such as alkyl phosphates, alkyl sulfates, and even fatty acids assemble into bilayer membranes if they contain 10 or more carbons in their hydrocarbon chains. In more recent work, Roberts (125) made similar observations with phospholipids. For instance, phosphatidylcholine with 12-carbon chains produces stable lipid bilayer membranes, while phosphatidylcholine with 8-carbon chains forms only micelles.

Cell membranes require fluid lipid bilayers for normal function, and if membranes undergo a phase transition to the gel state (for instance, at lower temperatures), the cell cannot function normally and often does not survive the fluid-gel phase transition. Primitive cell membranes presumably had the same requirement. The fluid state is regulated and maintained by variations in chain length, unsaturation, and, in certain prokaryotic cells, chain branching, and these factors should be taken into consideration when we discuss possible compositions of early membranes.

The sine qua non of the first lipid-like molecules is therefore hydrocarbons with sufficiently long chains. Until recently, it was believed that long-chain hydrocarbons were present in meteorites, and it followed that synthetic pathways were likely to have been available in the prebiotic environment. However, Cronin et al. (29, 30) found that the long-chain hydrocarbon content of the Murchison meteorite is in fact a terrestrial contaminant and that only cyclic aliphatic hydrocarbons appear to be present in small amounts. Mono- and dicarboxylic acids are relatively abundant (100 to 200 ppm), but chain lengths longer than 6 to 8 carbons are present only in trace quantities (83, 86).

Another possible source of hydrocarbons is chemical processing in the prebiotic environment. For instance, Fischer-Tropsch synthesis is an obvious possibility, and Nooner et al. (105) produced a variety of hydrocarbons and long-chain monocarboxylic acids by passing carbon monoxide and hydrogen gas over a hot catalyst (metallic iron at 200 to 400°C). Could such specialized conditions have been present on the prebiotic Earth? By the time the primary accretion of the primitive Earth was complete, most of the iron and other siderophiles were incorporated into the core. However, it is possible to imagine that volcanic regions may have incorporated small deposits of metallic iron so that volcanic gases such as carbon dioxide, carbon monoxide, hydrogen, and water vapor passing over the hot iron would provide naturally occurring Fischer-Tropsch conditions.

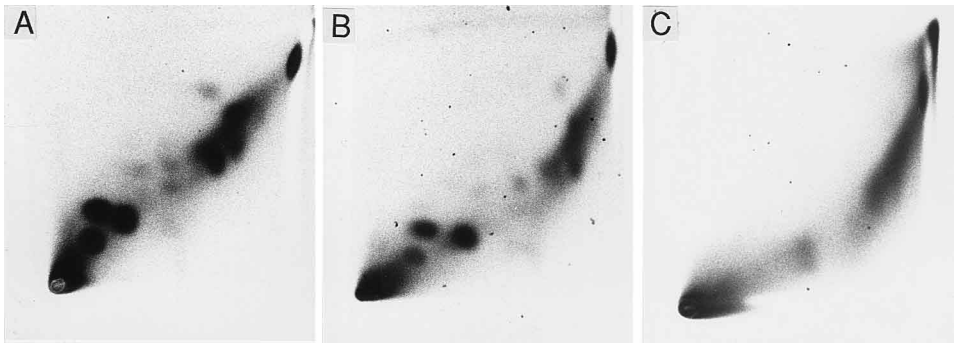

FIG. 9. Fluorescent signatures of organic extracts from three different carbonaceous chondrites. (A) Murchison meteorite. (B and C) Antarctic meteorites ALH84044 and LEW 8534. Aliquots (~500 mg) of the meteoritic powder were extracted at acidic pH ranges with sulfuric acid (0.1 N)-chloroform-methanol (2:2:1). The chloroform phase was dried and spotted on 20-cm silicic acid thin-layer chromatography plates, which were then developed in hexane-diethyl ether (4:1) (*y* axis) and chloroform (*x* axis). The images are negative prints showing fluorescence as darker regions. Nonpolar compounds such as pyrene, anthracene, and fluoranthene are at the upper right in the chromatograms, and more-polar compounds such as monocarboxylic acids are in the fluorescent region just above the origin at the lower left. The material shown in Fig. 10 was eluted from silicic acid scraped from this region of the Murchison plate (A).

An alternative approach to lipid synthesis was taken by Ourisson and Nakatani (116), who noted that molecular fossils commonly contain a variety of terpenoid and hopanoid derivatives. Terpenoids dominate the lipid composition of the *Archaea*, and the thermophilic archaebacteria are generally considered to resemble some of the earliest forms of microbial life. It is also true that most biological membranes contain some form of terpenoid or hopanoid, such as cholesterol, that contributes to the stability of the bilayer structure. From this, Ourisson and Nakatani proposed that acyclic polyprenols and their phosphate derivatives were the original lipids of membranes, with hydrocarbon chains synthesized by condensation reactions of isopentenols, derived from formaldehyde and isobutene. In other work, Ourisson has presented evidence that dipolyprenol phosphates containing geranyl chains (C_{10}) or farnesyl chains (C_{15}) are able to self-assemble into vesicular membranes.

In general, the evidence provided by molecular fossils and the structural role of terpenoids in the membranes of the *Archaea* is persuasive that terpenoids were an early lipid component of biological membranes. The argument that terpenoids played a role in prebiotic systems is less convincing, largely because an abundant source of precursors is problematic. Formaldehyde is high on the list of plausible prebiotic reactants, but isobutene is not. The synthesis of a 10-carbon geranyl polyprenoid phosphate would require the combination of relatively concentrated isoprenol, phosphate, and a catalytic surface, a seemingly improbable occurrence.

SELF-ASSEMBLY PROCESSES IN PREBIOTIC ORGANIC MIXTURES

To summarize the results described above, a prebiotic source of long-chain hydrocarbons is not obvious, nor is it easy to imagine how oxidized hydrocarbon derivatives such as fatty acids were produced even if hydrocarbons were abundant. Despite this reservation, the presence of membrane structures is not out of the question. We could even argue that for cellular life to begin, there must have been some process by which bilayers assembled from existing sources of lipid-like molecules. For a start, we might assume that the mixture of organic compounds in carbonaceous meteorites resembles components available on the early Earth, either through extraterrestrial infall or by synthetic processes occurring at the Earth's surface. The fact that amino acids are present in carbonaceous meteorites gave strong support to the idea that there were abiotic synthetic pathways to such molecules. We could therefore determine whether lipid-like molecules also occur in carbonaceous meteorites. If they are present, it would strengthen the case that membrane-like structures were present in the primitive environment.

For this reason, we have investigated several carbonaceous meteorites with respect to possible amphiphilic components, and I will describe results from the Murchison carbonaceous chondrite here (32, 39). The Murchison meteorite fell near Murchison, Australia, in 1969 and is the most intensively investigated source of meteoritic organic compounds. A kerogen-like insoluble polymer composed largely of covalently linked PAHs is the most abundant organic material (about 1% by mass), and a series of organic acids (including 10 to 20 ppm of amino acids) represents the most abundant water-soluble fraction. Aliphatic and aromatic hydrocarbons, ureas, ketones, alcohols, aldehydes, and purines are also present (see reference 29 for a review). A variety of amphiphilic molecules have been reported, including monocarboxylic acids up to 10 or so carbons long. PAHs are commonly found as components of the meteoritic organics, and their polar derivatives would presumably be sufficiently amphiphilic to participate in membrane formation.

Lipid-like organic components can be extracted by a two-phase chloroform-methanol-water system similar to that used to isolate membrane lipids from tissues (39). Two-dimensional thin-layer chromatography revealed a complex mixture of oxidized aliphatic and aromatic hydrocarbons (Fig. 9). When the organic acid fraction of the mixture was allowed to interact with the aqueous phases, one class of minor components clearly was capable of forming membrane-bounded vesicles (Fig. 10). The vesicles responded osmotically to sodium chloride or sucrose additions and could maintain gradients of a negatively charged fluorescent dye (pyranine).

Electron microscopic examination of thinly sectioned material showed obvious membranes surrounding accumulations of nonmembranous material (Fig. 11). Under high magnification, the membranes appeared to be trilaminar structures typical of lipid bilayer membranes (Fig. 11, inset). Freeze-fracture images showed fracture planes as well, confirming the structures observed in thin sections by transmission electron microscopy.

By using mass spectrometry, we determined that one of the components was nonanoic acid, a nine-carbon carboxylic acid. Nonanoic acid has too short a chain to form stable bilayers capable of maintaining solute gradients, but under conditions

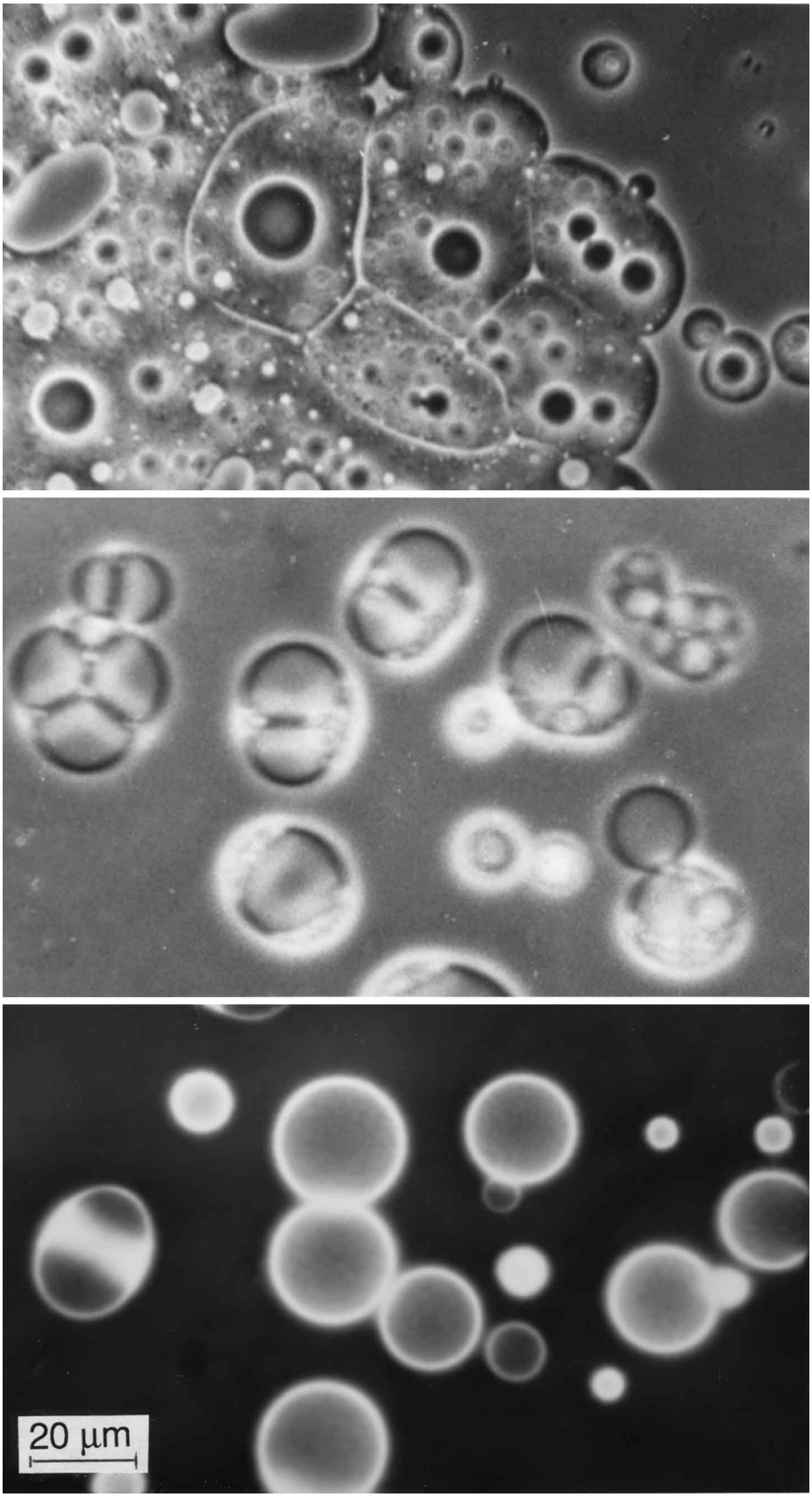

FIG. 10. Formation of membranous vesicles from mixtures of meteoritic amphiphilic compounds. Eluate from plate A of Fig. 9 was dried on a standard glass microscope slide and allowed to interact with a dilute alkaline buffer (10 mM $NaCO_3$). Under these conditions, the aqueous phase penetrated the dried extract within minutes (top) and vesicle formation ensued over a 30-min period (center). The vesicles contained fluorescent compounds in their membranes, which could be visualized by epifluorescence microscopy with 400-nm blue light for excitation (bottom). If a fluorescent ionic dye such as pyranine was included during the initial hydration, it was captured by the vesicles (not shown).

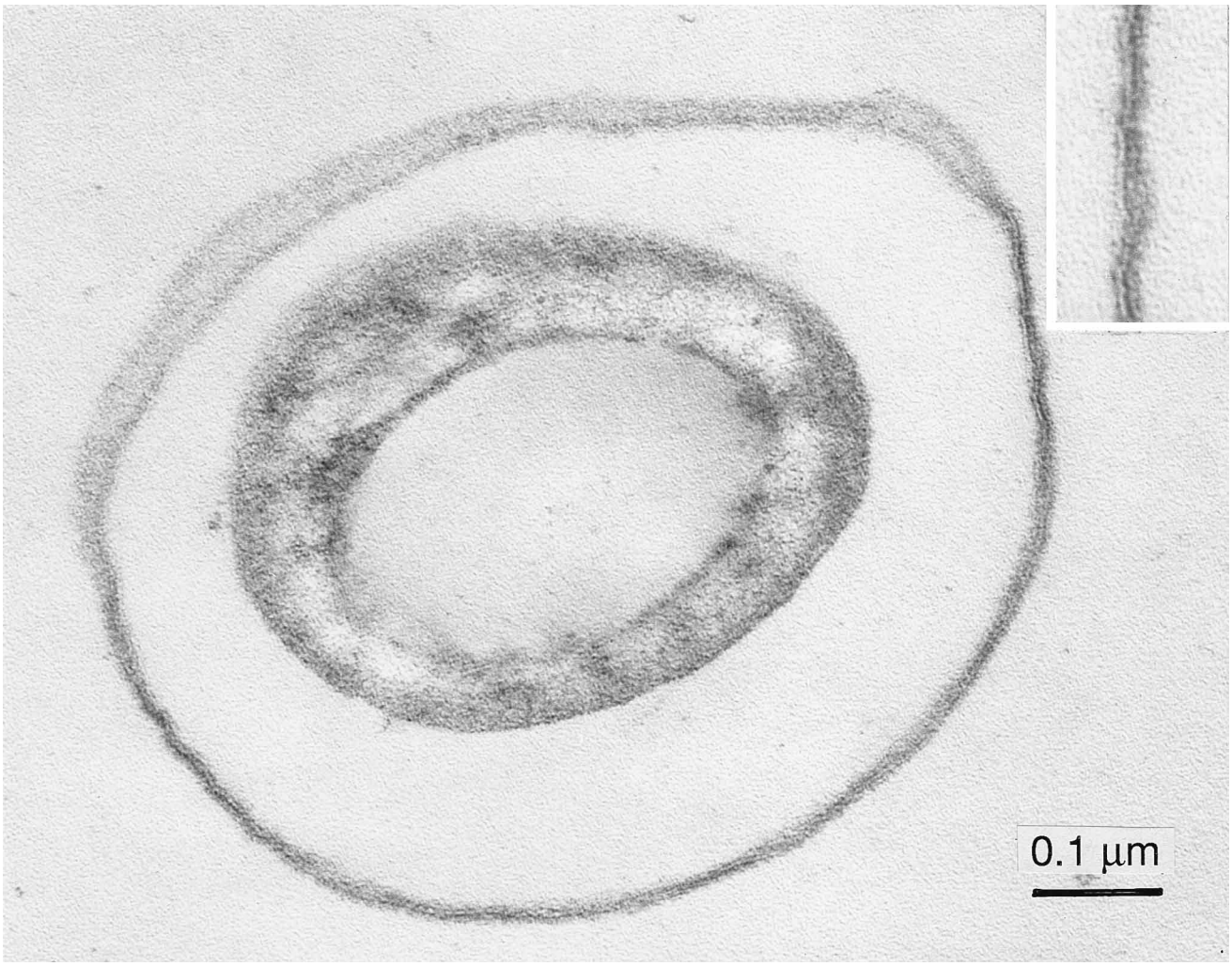

FIG. 11. Transmission electron micrograph of a vesicle produced by sonication of the sample shown in Fig. 10. Virtually all of the vesicles have a membrane surrounding an internal mass of nonmembranous material. At higher magnification, the outer membrane shows the trilaminar structure characteristic of bilayer membranes. Freeze-fracture images reveal clear fracture planes, confirming that bilayers are present. Reprinted from reference 39 with permission.

of neutral pH and a high concentration of nonanoate (100 mM), membrane structures were readily observed by light microscopy (Fig. 12).

From these results, we may conclude that surface-active compounds capable of membrane formation are present in carbonaceous meteorites in a fraction corresponding to organic acid regions on thin-layer chromatography plates. The membrane structures can be visualized by light microscopy, by thin sectioning of osmium-fixed specimens for electron microscopy, and by freeze-fracture methods. The membranes are approximately 10 nm thick and show a trilaminar staining pattern with a 5.5-nm spacing between the densely stained lines, as expected for a bilayer of some amphiphilic material. The fact that they manifest fracture planes by the freeze-fracture technique is also consistent with bilayer organization. The evidence from mass spectrometry suggests that the membrane-forming amphiphiles are probably mixtures of monocarboxylic acids and polar aromatic compounds that have amphiphilic properties. More detailed analysis has not yet been possible because of the microscopic quantities available.

ENCAPSULATION OF MACROMOLECULES BY BILAYER VESICLES

It seems reasonable to speculate that the origin of cellular life took place in a concentrated mixture of chemical components that had already established simple metabolic pathways and perhaps a catalyzed polymerization of primitive genetic material. For lipid bilayer vesicles to capture the large molecules involved and thereby produce the first cells, a robust encapsulation mechanism would be required. A membrane that provides an encapsulated environment would also tend to exclude polar and ionic solutes, as well as macromolecules. If encapsulation took place, a reversible process must be possible by which the bilayer barrier was first broken, allowing the entry of large molecules, and then resealed.

Most liposome encapsulation procedures are highly technical and involve processes like detergent solubilization, sonication, and extrusion through polycarbonate filters. However, one encapsulation mechanism that has the potential to function in the prebiotic environment depends on the fact that when liposomes are dried in the presence of macromolecules,

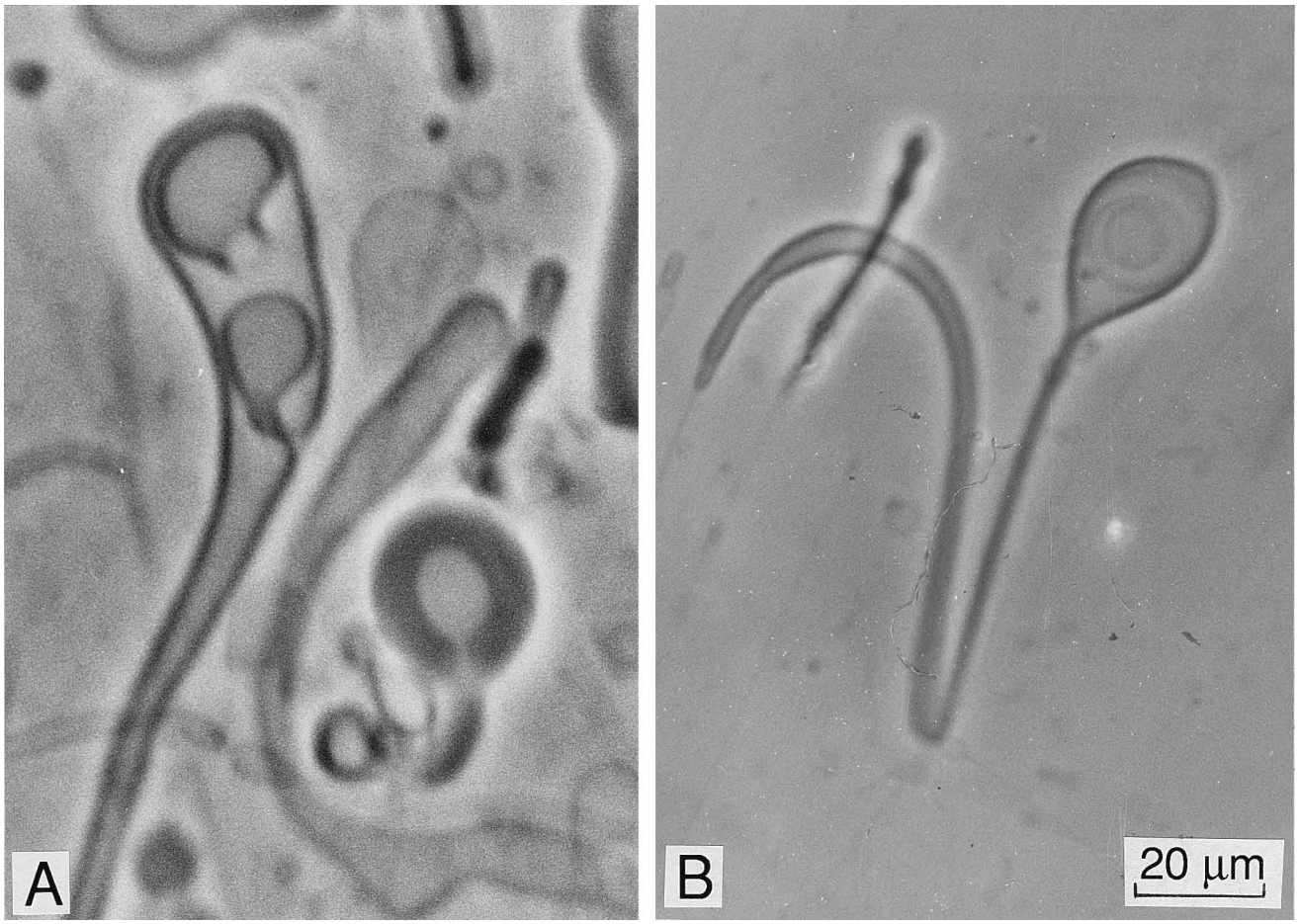

FIG. 12. Comparison of membranous structures produced by nonanoic acid (A) and amphiphilic organic material extracted from the Murchison carbonaceous meteorite (B).

they tend to fuse into multilayered structures that "sandwich" the solutes, as shown in Fig. 13 (34, 136). The macromolecules are then captured upon rehydration, when the lipid layers reseal into vesicles. It is not difficult to imagine hydration-dehydration cycles occurring in intertidal zones, and it follows that encapsulated systems of macromolecules may have been reasonably common.

The last question to be discussed is whether bilayer formation could have been driven prebiotically by an energy-dependent process. A model system for such a reaction is the production of lipid-like amphiphiles from precursor molecules, by using energy stored in the precursors or delivered to the precursors from some external source. One example is the production of membrane structures when a lysophospholipid such as lysolecithin is acylated by acyltransferase, with acyl coenzyme A as a source of the acyl group and as a source of energy to drive the reaction (55). Bachman et al. have developed simpler systems that produce membranous structures at the expense of stored chemical energy and have introduced the term "self-reproduction" to describe such systems (3). That is, the membranes themselves catalyze the reaction by which their components are produced. Such systems build on the fact that single-chain amphiphiles readily assemble into bilayer vesicles, and Walde et al. (154) showed that membrane vesicles can undergo growth and a kind of reproduction. The system depends on the hydrolysis of nonmembranous precursor molecules, such as fatty acid esters and anhydrides, to fatty acid-soap mixtures that can form bilayer vesicles at certain pH ranges. Wick et al. (164) used light microscopy to monitor vesicle growth in a system of giant liposomes and found that vesicles large enough to be observed grew over a 6-h period from an average diameter of approximately 1 μm to a diameter between 4 and 6 μm. Although the oleic anhydride is not a plausible prebiotic substrate, the results of Walde et al. (154) provide an interesting model system by which bilayer synthesis and growth can be readily investigated.

MEMBRANE PERMEABILITY AND EARLY BIOENERGETICS

Although membranes define all living cells, the membrane also limits access to nutrients and energy sources. As noted above, the first living cellular systems were unlikely to have evolved specialized membrane transport systems, and it is interesting to consider how early cells overcame the barrier. To give a perspective on permeability and transport rates, we can compare the fluxes of relatively permeable and relatively impermeable solutes across contemporary lipid bilayers. The measured permeabilities of lipid bilayers to small, uncharged molecules such as water, oxygen, and carbon dioxide are approximately 10^9-fold greater than the permeability to ions. For instance, the permeability coefficient of water is approximately

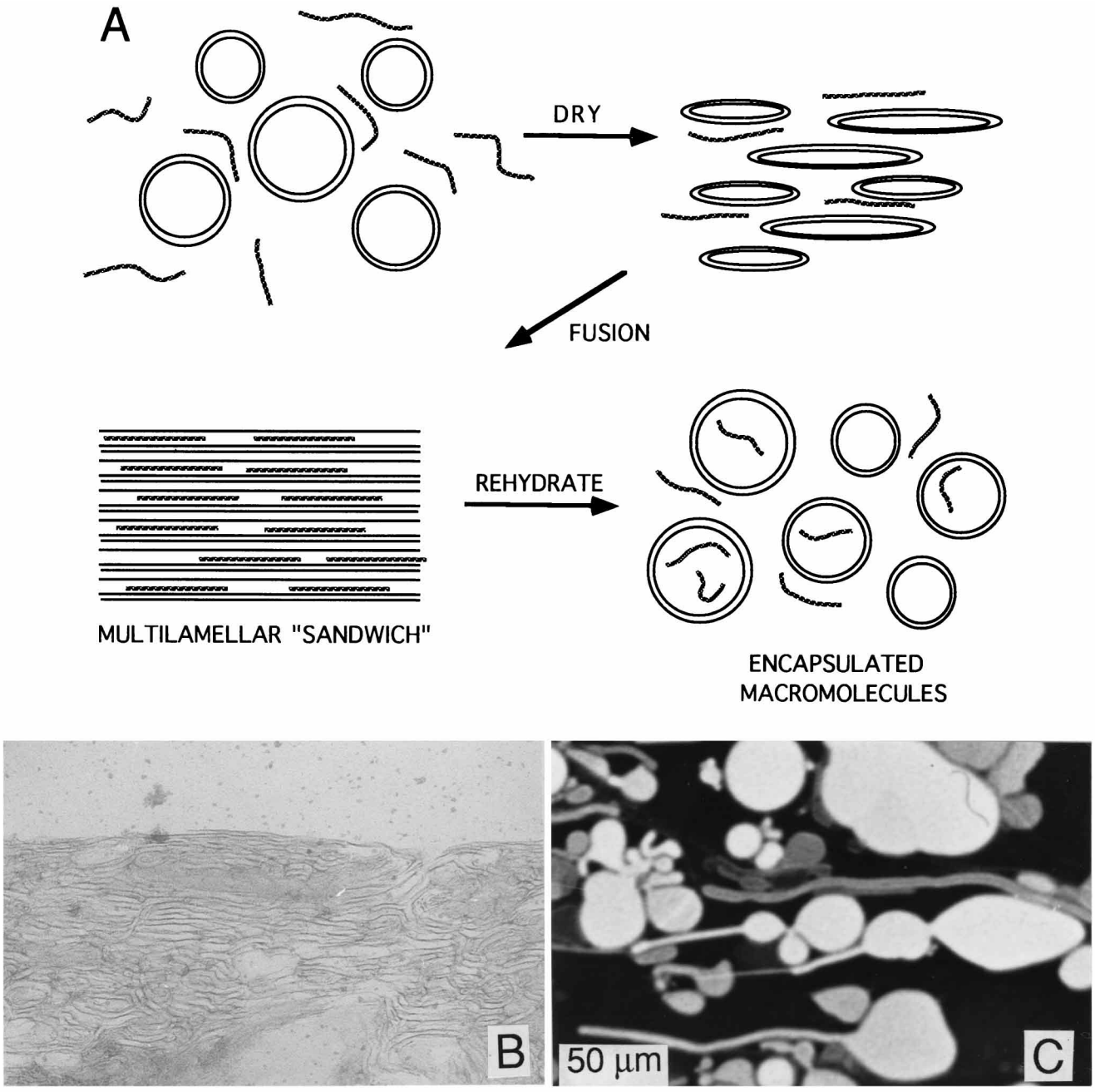

FIG. 13. Encapsulation of macromolecules during a drying-wetting cycle. (A) If liposomes are mixed with soluble proteins or nucleic acids and dried, the liposomes fuse to produce a multilamellar structure in which the macromolecules are "sandwiched" between lipid layers. Upon rehydration, vesicles form that encapsulate up to half of the soluble macromolecules. (B) Electron micrograph of a 2:1 (wt/wt) mixture of dioleoylphosphatidylcholine-salmon testis DNA after drying, showing the fused multilamellar structure. (C) Fluorescence micrograph of the lipid-DNA mixture following rehydration. Acridine orange was used to stain the DNA captured in large lipid vesicles.

10^{-3} cm s^{-1} and the permeability coefficient of potassium is 10^{-12} cm s^{-1}. These values mean little by themselves but make more sense when put in the context of the time required for exchange across a bilayer. Measurements show that half the water in a liposome exchanges in milliseconds whereas potassium ions have half-times of exchange measured in days (9).

We can now consider some typical nutrient solutes like amino acids and phosphate. Such molecules are ionized, which means that they would not readily cross the permeability barrier of a lipid bilayer. Permeability coefficients of liposome membranes to phosphate and amino acids were recently determined (21) and found to be in the range of 10^{-11} to 10^{-12} cm s^{-1}, that is, in the same range as other ions. From these values, one can estimate that if a primitive microorganism depended on passive transport of phosphate across a lipid bilayer composed of a typical phospholipid, it would require several years to accumulate sufficient phosphate to double its DNA content or pass through one cell cycle.

One solution to this conundrum is simply to shorten the chain length of the lipids composing the bilayer. It has been shown that shortening phospholipid chains from 18 to 14 carbons increases the permeability of the membrane to ions nearly 10^3-fold (120). The increased permeability is apparently due to transient transmembrane defects that become increasingly common as the bilayer thins. In fact, bilayers composed of 10 carbon lipids are so permeable that ion gradients cannot be maintained for more than a few seconds. Intermediate-chain-length lipids therefore provide a barrier sufficient to encapsulate macromolecular catalysts while allowing smaller ionic nutrients access to the interior.

In recent work (20), we have taken advantage of this effect to demonstrate directly that a polymer can be synthesized by an encapsulated enzyme system that depends on transmembrane transport of substrate and chemical energy (Fig. 14). Polynucleotide phosphorylase (PNPase) uses nucleoside diphosphates such as ADP as substrates to synthesize polyribonucleotides. An advantage of PNPase is that it does not require primers or templates, and as early as 1976 Oparin et al. attempted to show that PNPase associated with coacervates could synthesize RNA (108). In our work, encapsulation of the enzyme was carried out by dehydration of dimyristoylphosphatidylcholine (DMPC) vesicles in the presence of the enzyme followed by rehydration, a process that produces liposomes containing the enzyme as described above. Any PNPase remaining outside of the liposomes was removed by gel filtration.

ADP was then added as a potential substrate for the enzyme. To demonstrate that ADP could cross the bilayer, the release of encapsulated ADP from DMPC vesicles was first measured at three different temperatures. We found the permeability coefficient to be 2.6×10^{-10} cm s^{-1} at 23°C, sufficient to supply the enzyme with ADP. The half-time of release above and below this temperature was increased approximately twofold, presumably because the DMPC has a phase transition at 23°C that produces increased numbers of transient defects. We ran the reactions at 23°C, but we obtained equivalent results at 37°C, because the lower permeability of the substrate was balanced by increased enzyme activity at the higher temperature.

RNA synthesis was measured in two ways. In the first, increased ethidium bromide fluorescence was monitored as RNA was produced by the enzyme. We found that the reaction rate with encapsulated enzyme was only about 20% of that with the free PNPase, suggesting that the DMPC bilayer, even though orders of magnitude more permeable than a lipid bilayer composed of 18-carbon lipids, still represented a partial barrier to substrate permeation. Poly(A) synthesis was also monitored by polyacrylamide gel electrophoresis. In this experiment, to control for any PNPase bound to the outer surface of the liposomes, a protease was added. The protease completely inhibited RNA synthesis by the free enzyme, but the encapsulated PNPase was protected, with significant RNA synthesis observable. RNA could be visualized in the liposomes by fluorescence microscopy of the ethidium bromide.

These results provide a helpful perspective on substrate transport by primitive forms of life. On the early Earth, a variety of amphiphilic hydrocarbon derivatives could self-assemble into bilayer boundary structures. However, it is not necessary to think that the amphiphiles were of the same length as those composing contemporary membranes. Instead, membrane-forming amphiphiles with 12- to 14-carbon chains, modeled here by DMPC, would produce bilayers that are permeable enough to allow the passage of ionic substrates required for polymerization of macromolecules such as RNA yet maintain those macromolecules within a boundary. Encapsulated catalysts and information-bearing molecules would thus have access to nutrients required for growth processes. Furthermore, specific groupings of macromolecules would be maintained, rather than drifting apart. This provides the significant advantage of allowing true selection to occur, which would be absent in free molecules.

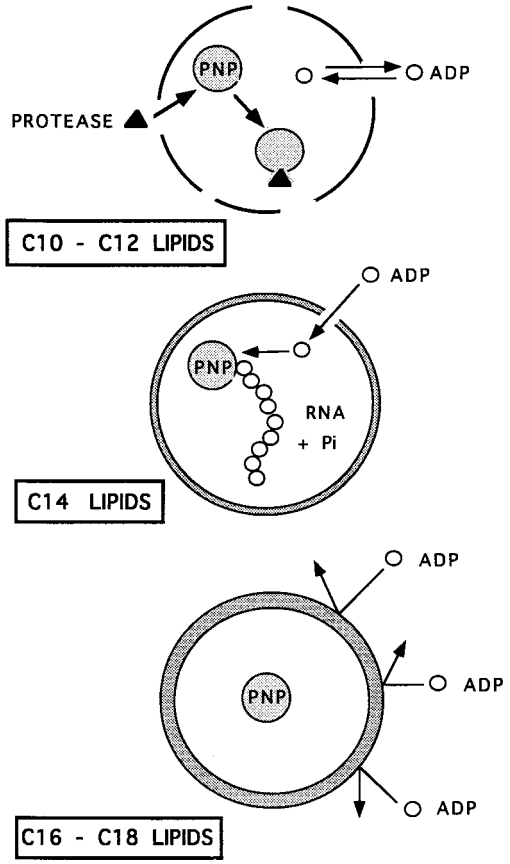

FIG. 14. Substrate transport to an encapsulated polymerase. Polynucleotide phosphorylase (PNP) can use nucleoside diphosphates such as ADP to generate RNA (polyadenylic acid). PNP was encapsulated in liposomes of different chain lengths (20). If the lipid chains were relatively short (C_{10} to C_{12}), the bilayers were highly permeable due to large numbers of fluctuating transient defects in the bilayer. ADP could potentially reach the encapsulated PNP, but the enzyme could not be protected from external damaging agents such as the protease shown in the top figure. The high permeability also allowed product to escape. On the other hand, permeation of ADP across bilayers with 16 to 18 carbons was too slow for the ADP to be a substrate for the enzyme. However, with an appropriate choice of lipid, in this case the 14-carbon DMPC, the enzyme could be supplied with substrate at a sufficient rate while still being protected against protease activity.

Luisi and coworkers have found a way to couple membrane growth to the synthesis of RNA within vesicles. In one such system, polynucleotide phosphorylase was first trapped in liposomes composed of oleic acid, and ADP was then added (155). Because the oleic acid/oleate membranes are considerably more permeable than phospholipid bilayers, the ADP readily permeates the membrane and can be used as a substrate by the enzyme. After a lag period of 4 days, polyadenylic acid began to be synthesized, at which point oleic anhydride was added. For the next 30 h, the oleic anhydride was hydrolyzed to oleic acid, which was incorporated into the vesicle membranes, and polyadenylic acid was synthesized at the same time. This represents the first model system to incorporate catalyzed growth

through polymerization of an encapsulated macromolecule that is accompanied by growth of the encapsulating membrane.

To determine whether a template-driven reaction can function within the vesicle environment, Oberholzer et al. (106) captured the components of a PCR amplification within liposomes and showed that small amounts of DNA could be produced. This result was enlightening in several ways. First, ordinary phospholipid is sufficiently stable to maintain membrane integrity even at PCR temperatures as high as 95°C. Second, the result clearly demonstrated the limitations of membrane-encapsulated reactions that must be overcome before we can take further steps toward a model system with properties of the living state. That is, the liposomes were prepared with a phospholipid that forms relatively impermeable bilayer membranes. This means that eight different solutes must be captured in any one liposome if a successful reaction is to take place, including the polymerase, a DNA template, two specific primers, and four nucleoside triphosphates, as well as magnesium ion. By calculation, the authors determined that only a very small fraction of liposomes would capture all eight solute species. Furthermore, a typical liposome in this preparation had a measured volume of 3.3×10^{-18} liter. At 1.0 mM concentrations, only 2,000 molecules of each substrate would be captured, barely sufficient to produce 10 or so double-stranded DNA product molecules (369 bp) in each liposome. To take this system further, it will be necessary to establish a mechanism by which externally added substrates have access to the encapsulated enzymes.

CONCLUSIONS AND FUTURE DIRECTIONS

I now summarize the primary points of this review and suggest where further research could be usefully undertaken.

(i) It is virtually certain that organic material was delivered to the Earth's surface as extraterrestrial infall during the Archean era. The certainty arises primarily from the fact that such infall continues today in the form of meteoritic material that is available for analysis. It is also reasonable to assume that there were synthetic processes by which carbon and nitrogen were first chemically activated by high-energy sources such as UV light and electrical discharge, followed by condensation to more complex compounds such as amino acids, purines, and simple carbohydrates. As a result, we can assume with considerable confidence that a source of relatively simple organic molecules was available on the early Earth and that such molecules accumulated in oceans, lakes, and perhaps marine hydrothermal systems. It is also certain that accumulated organic material disappeared at different rates by a second series of reactions, including hydrolysis, photochemical degradation, and pyrolysis. A primary question here is to determine the relative amounts of organic input by infall and terrestrial synthesis and the kinetics of the turnover process that balanced synthesis and degradation.

(ii) It seems unlikely that living systems originated through reactions in dilute solution of simple solutes such as amino acids, carbohydrates, and purine bases. Instead, a variety of concentration processes which brought otherwise dilute reactants into close contact would be involved. Three such processes include simple drying of shallow pools at water-land interfaces, adsorption to polar or charged mineral surfaces, and self-assembly of nonpolar and amphiphilic organic compounds into films, micelles, bilayers, and oil droplets.

(iii) Energy must have been captured by the molecular systems in a variety of forms. The simplest energy capture would be through condensation reactions driven by dehydration and heating of dried films, and abundant research has demonstrated that polymerization can result from such processes. A second form of energy capture would be through redox reactions; examples include iron mineral surfaces and conditions associated with the geothermal processes related to hydrothermal vents. A third abundant energy source would be light, assuming that suitable pigment systems were available. At first, the pigments would be components of the prebiotic environment which were incorporated into molecular aggregates by self-assembly. Only later would specific pigment molecules become products of biosynthetic processes. Examples of pigments that would probably have been present in the environment include ferrous iron and iron complexes with organic compounds, and PAHs and their derivatives.

(iv) Relatively long-chain hydrocarbons and their derivatives must have been present in the organic inventory of the early Earth. Cellular life arose at some point, and it could do so only if hydrocarbons were available for membrane assembly.

(v) Finally, given that bilayers and pigment systems appeared at some point in the origin and evolution of early life, it is reasonable to expect that ion gradients would be available as potential sources of energy. This energy source could be used to drive the transport of solutes, just as it does today, and at a later time coupling mechanisms that used the energy of the gradients to drive chemiosmotic reactions presumably evolved.

In terms of future research directions, it is interesting to consider whether it might be possible to assemble a true replicating system of encapsulated polynucleotides that can undergo cell-like growth. From the evidence reviewed here, it is clear that macromolecules such as polymerases can be incorporated into self-assembled vesicles and that encapsulated polymerases can use chemically activated monomers to support nucleic acid synthesis within the vesicles. The next step is to somehow bypass the requirement for protein enzymes as catalysts, because they are not reproduced in the defined system. Instead, the catalyst should incorporate both genetic information and the polymerase activity, for instance, a ribozyme-like catalyst. Finally, a process by which membrane components are added to the vesicles must also be part of the system, with some form of regulatory coupling between the growth of the internal molecules and growth of the membrane. In practice, there is still no way to deal simultaneously with all of these requirements. However, a few years ago it would have been inconceivable that we would soon reach a point at which intravesicular polymerization of nucleic acids became a reality. It seems likely that a laboratory version of an encapsulated replicating system of molecules is a real possibility in the near future.

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